Phosphoramidite ligands and artificial metalloenzymes in enantioselective rhodium-catalysis

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Phosphoramidite Ligands and Artificial Metalloenzymes in Enantioselective Rhodium-Catalysis:

Old Challenges and New Frontiers

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Contents

Chapter 1
Introduction

1.1 Catalysis: classification and characteristics

The word catalysis derives from the Greek noun κατάλυσις, related to the verb καταλύειν, meaning *to annul* or *to untie* or *to pick up*.[1] Although Faraday is considered to have been the first scientist to study a catalytic reaction (the reaction of H_2 and O_2 on platinum), this term was first introduced by Berzelius in 1835. However, it was Ostwald who in 1902 defined catalysts as reaction accelerating agents.[2]

Catalysts are defined as species that participate in the chemical reaction providing an alternative reaction pathway with lower Gibbs energy of activation (and therefore a different reaction mechanism) and result unchanged at the end of the transformation. This will cause an increase in the reaction rate without altering the equilibrium composition of the system (Figure 1.1).[3]

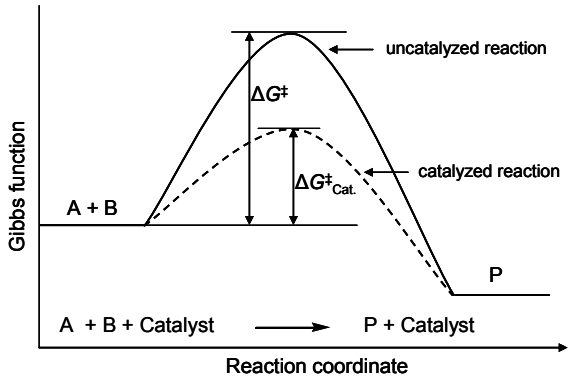


Figure 1.1 *The presence of a catalyst causes a decrease of the activation energy*

The action of a catalyst can be explained in terms of:

- Stabilization of the highly reactive and unstable activated complex.
- Ability of bringing the reagents in proximity and/or forcing their correct orientation, with consequent change in entropy.
- Favoring a specific pathway over competing and undesired ones.

Enzymes are probably the most famous and efficient catalysts with reaction rate enhancements up to 10^{17} (k_{cat} / k_{uncat}).[4]

As depicted in Figure 1.2, from a physical state point of view, catalysis is divided in homogeneous (catalyst and reagents are present in the same phase) and heterogeneous (catalyst and reagents are present in different phases).

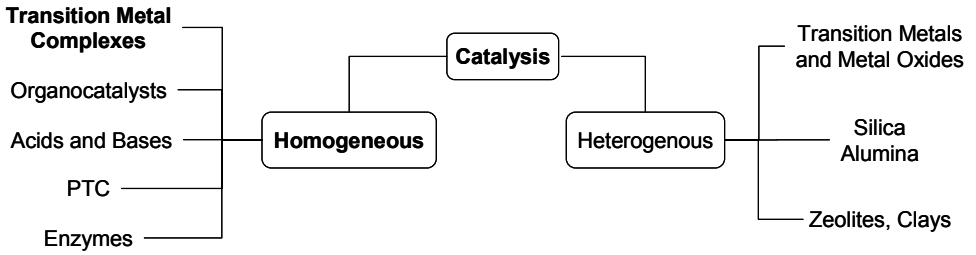


Figure 1.2 *Catalysis: a classification*

Heterogeneous catalysts have the advantage of being easily separated from the reaction mixture, often being stable even under harsh conditions and the processes in which they are involved can be easily scaled up, which is important for industrial applications. However, homogeneous catalysts have a broader scope in organic synthesis due to some of their key features, such as:

- Operational under milder reaction conditions.
- Reaction reproducibility.
- Accessible mechanistic insight.
- Easier modification of the catalyst properties.
- Higher selectivity.

According to the nature of the catalyst, homogeneous catalysts can also be divided into nucleophilic (Lewis bases, Brønsted bases, biological), electrophilic (Lewis acids, Brønsted acids) and coordination catalysts (transition metal complexes).

Enzymes are still considered to be the most efficient homogeneous catalysts due to their high activity and specificity. As a consequence, biocatalysis recently started to find an increasing number of applications in organic chemistry.[5] However, in the last decades huge advances in terms of activity and selectivity have been achieved in transition metal catalysis, resulting in a large diversity of possible reactions (Figure 1.3), some of which can only be performed using coordination catalysis.[6]

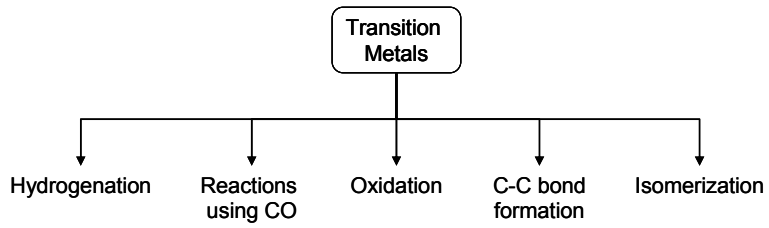


Figure 1.3 *Classes of reactions studied using transition metal complexes*

1.2 Homogeneous catalysis using transition metal complexes

Transition metal catalysts consist of a metal center complexed with appropriate ligands. A different transformation often requires a different metal center that is responsible for the catalytic activity, due to its direct interaction with the substrate. This interaction provides the alternative pathway with lower Gibbs energy of activation, which allows the reaction to take place (*vide supra*). However, it is the ligand which modulates the properties of the catalysts and will determine the level of selectivity that can be obtained due to its electronic and steric properties.[7] In this respect, it is noticed that the most widely exploited complexes contain the following metals and ligand classes:[8]

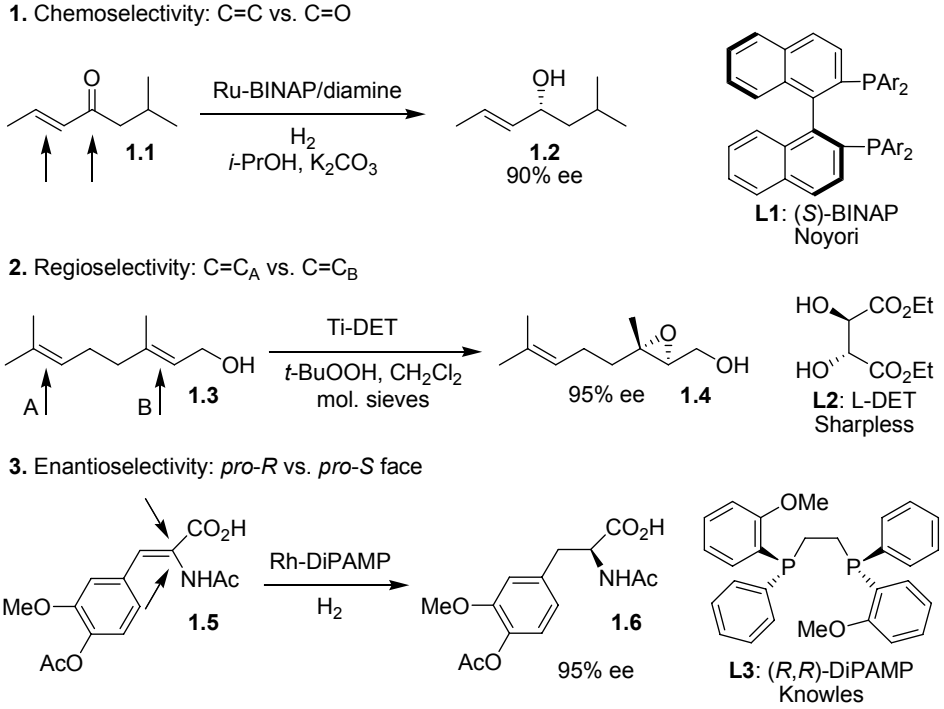
- Pd, Rh, Ru, Ir, Os, Pt and Ni are generally complexed with tertiary P and/or N containing ligands (but also Cp and CO).
- Ti, Zn, Co, Mn and Cu are generally complexed with O and/or N containing ligands.

The use of transition metal catalysts allows unprecedented levels of selectivity control in terms of:

- Chemoselectivity: discrimination between different functional groups.
- Regioselectivity: discrimination between equivalent functional groups or atoms.
- Enantioselectivity: discrimination between the two faces of a prochiral substrate or enantiotopic atoms and groups, leading to a chiral compound.[9]

Representative examples, in which these selectivity issues have been addressed, can already be found in the pioneering studies of Knowles (on olefin hydrogenation),[10] Noyori (on ketone hydrogenation)[11] and Sharpless (on olefin epoxidation),[12] who were awarded the 2001 Nobel Prize in Chemistry for their achievements in asymmetric catalysis using chiral transition metal complexes (Scheme 1.1). These studies marked a new era in coordination chemistry, in which metal catalysts, due to their activity and selectivity, started to be considered *chemists' enzymes*, thereby reducing the gap between chemo- and biocatalysis.

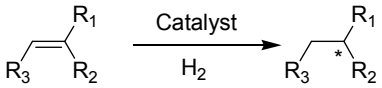
Catalytic asymmetric synthesis (or asymmetric catalysis) is defined as an enantioselective transformation controlled by a chiral catalyst, (e.g. a metal complexed with chiral ligands). Control over the absolute stereochemistry of a transformation is one of the most current and most studied aspects in homogeneous catalysis and it has been classified as one of the major challenges in contemporary organic synthesis.[6] Therefore, the use of a catalytic amount of a chiral complex capable of transferring its chirality to prochiral substrates during a reaction appears to be an appealing approach for the preparation of enantiopure compounds (*vide infra*).



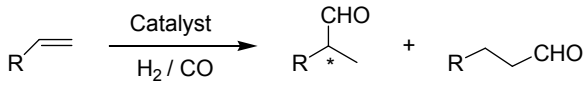
Scheme 1.1 *Examples of selectivity issues addressed by transition metal-catalysis*

New methodologies, in order to be synthetically appealing and meet the modern environmental and sustainability requirements, should not only be selective but also atom efficient.[13] In the ideal case, readily available starting materials are transformed into products without the use of additives, in the presence of a small amount of catalyst, and without the production of any waste. Increasing efforts have been devoted to achieve this target and a small number of metal-catalyzed transformations, such as hydrogenation and hydroformylation reactions, already meet these requirements (A and B, Scheme 1.2). Catalytic metathesis is another excellent example of efficiency and selectivity in C-C bond formation combined with atom economy (C, Scheme 1.2).[14] The achievements obtained and the future implications of this transformation in organic synthesis have been recently recognized by awarding Chauvin, Grubbs and Schrock the Nobel Prize for Chemistry in 2005.[15]

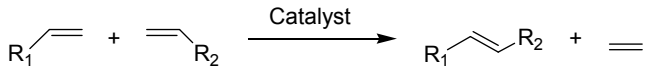
A. Hydrogenation:



B. Hydroformylation:



C. Metathesis:



Scheme 1.2 *Examples of atom economy conversions*

After all these considerations, it would appear that transition-metal catalysts are the perfect answer for modern organic synthesis issues in terms of reactivity, selectivity and atom economy. Their use for the preparation of highly valuable chiral intermediates seems to open a new perspective for the industrial preparation of pharmaceuticals, agrochemicals and functional materials.

Continuous progress is made in the understanding of existing metal-catalyzed reactions and in the discovery of new methodologies and applications. Particular attention is devoted to the study of Pd, Ru and Rh based catalysts because of their versatility and to the design of new chiral ligands (most of which are phosphorus based)[16] able to improve performance, stability and practical utility of the corresponding catalysts.

1.3 Chiral intermediates

Enantiopure intermediates have an estimated fraction of 15% of the market for the chemical industry. About 80% of the active compounds that pharmaceutical companies produce are chiral, and it is estimated that this fraction will increase. Moreover, the regulations concerning chiral active pharmaceutical ingredients impose the targeted synthesis of one stereoisomer, because of the possible different bioactivity of the opposite enantiomer.[17] Enantiomerically pure compounds also showed to be more effective in agriculture compared to the corresponding racemates, reducing the quantities used with a positive economic and environmental impact.[18]

As depicted in Figure 1.4, there are many different approaches for the preparation of enantiopure compounds, the most obvious being the chemical modification of enantiopure compounds derived from resources such as fermentation and agriculture. A disadvantage of this approach is the limited diversity of the starting

materials and the often difficult accessibility of the opposite enantiomer.[19] The resolution of racemates by crystallization is another important and widely used method to obtain enantiopure compounds in particular on industrial scale.[20] Moreover, the use of (dynamic) kinetic resolution allows to obtain a theoretical yield of 100% upon racemization of the "undesired" enantiomer.[21]

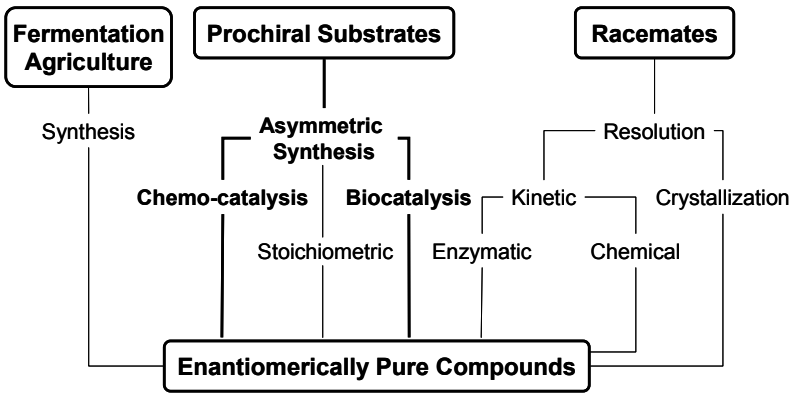


Figure 1.4 *Routes to enantiopure compounds*

Asymmetric synthesis may be carried out with the use of stoichiometric amounts of chiral reagents or auxiliaries.[22] However, catalytic asymmetric synthesis has clear advantages over the reagents and auxiliaries approach since a catalytic amount of chiral material can produce large quantities of enantiomerically enriched or enantiopure products. Therefore, the chirality is amplified rather than transferred.

At industrial level, survey projects estimated that in 2005 the worldwide revenues on chiral products had reached $9.5 billion.[23] The contribution of the different methodologies indicated a decrease of 6% compared to 2002 in the use of traditional technologies (fermentation and agriculture, separation and resolution) in favor of chemo-catalysis (from 35% to 36%) and biocatalysis (from 10% to 15%). The use of catalysis in industrial applications, both chemical and enzymatic, is supposed to increase further in the coming years.[23]

1.4 Rh-catalyzed asymmetric hydrogenation

Asymmetric hydrogenation continues to be the most widely applied enantioselective chemo-catalytic technology for the industrial manufacture of pharmaceutical intermediates.[24] This can be attributed to the fact that the reaction is clean (only requiring solvent, substrate, hydrogen and a small amount of catalyst) and is readily scaled-up. It was also the first catalytic asymmetric technology to be operated at industrial scale (namely the use of Rh-DIPAMP (**L3**) in the L-DOPA process).[10b] It has been estimated that at the moment 22 enantioselective chemo-catalytic processes are being operated on an industrial

scale and that 11 of these processes are asymmetric hydrogenations involving the use of rhodium, ruthenium and iridium catalysts.[25] A number of representative examples of Rh-catalyzed asymmetric hydrogenation processes and related ligands are depicted in Figure 1.5.

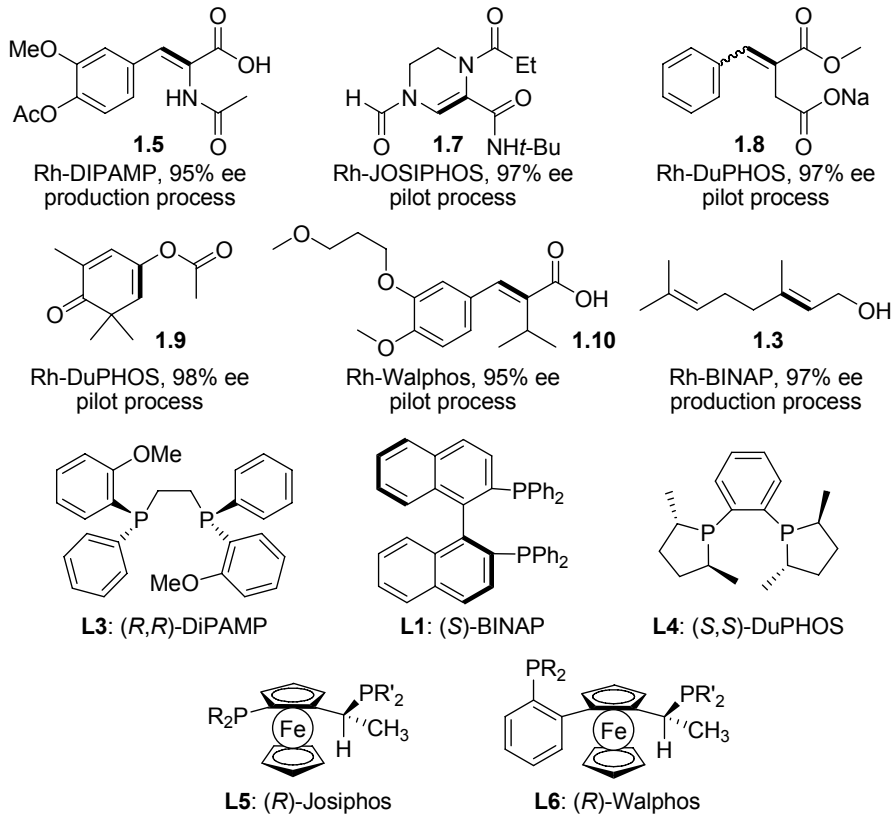
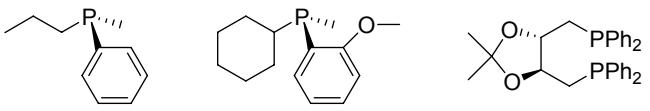


Figure 1.5 *Selected examples of production and pilot scale Rh-catalyzed asymmetric hydrogenation processes*[26]

The first steps toward modern homogeneous transition metal-based asymmetric catalysis were made 40 years ago, when Wilkinson and coworkers reported the first phosphorus based Rh-catalyst for the hydrogenation of alkenes, $[RhCl(PPh_3)_3]$.[27] Two years later, Horner and coworkers reported the preparation of stable chiral phosphines.[28] In the same year, Horner and Knowles and their coworkers reported the first use of monodentate phosphorus based ligands in Rh-catalyzed hydrogenation reactions.[10a,29] Only 15% ee was achieved in the hydrogenation of α-phenyl acrylic acid using **L7** (Figure 1.6). Nevertheless, these first attempts stimulated the creativity and the enthusiasm of numerous scientists in developing new and improved catalytic systems, giving shape not only to the field of Rh-catalyzed asymmetric hydrogenation, but to the whole area of asymmetric

transition metal catalysis as we know it today. Only few years later, in the early seventies, both CAMP[30] (**L8**, monodentate P-chiral ligand) and DIOP[31] (**L9**, bidentate with chiral backbone) were introduced and tested in the hydrogenation of α-amino acid precursors (Figure 1.6).



L7 **L8**: CAMP **L9**: DIOP

Figure 1.6 *First chiral phosphorus ligands developed*

Interestingly, the two ligands afforded comparable results in the hydrogenation of (*Z*)-2-(acetamido)cinnamic methyl ester (**1.11**, **L8:** 88% ee, **L9**: 70% ee). The use of DIOP (**L9**) demonstrated that it was not necessary to have a chiral phosphorus center in order to induce high enantioselectivity, with clear synthetic advantages. Moving the chirality from the phosphorus to the backbone favored not only the development of more and diverse ligands but also the synthesis of more and more bidentate ligands. They started to dominate the field especially after the implementation of DiPAMP (**L3**) in the industrial process of L-DOPA (Figure 1.5), as previously mentioned. In the early 90's, another step forward in the field of Rh-catalyzed enantioselective hydrogenation was made by the appearance of DuPHOS (**L4**) with showed unprecedented ability in inducing enantioselectivities up to 99% ee for a wide range of substrates.[32]

Historically, the most studied substrates in Rh-catalyzed asymmetric hydrogenation are α-dehydroamino esters, in particular (Z)-2-(acetamido)cinnamic (**1.11**) and 2-(acetamido)acrylic (**1.12**) methyl ester (Figure 1.7). The hydrogenation of this class of substrates leads to α-amino acid derivatives which are industrially interesting compounds (*vide infra*).

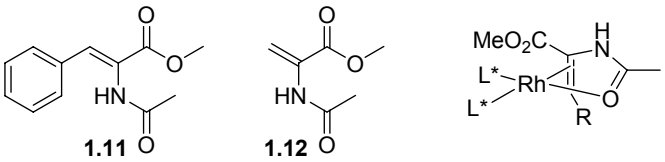


Figure 1.7 *Dehydroamino acid derivatives **1.11** and **1.12** are standard substrates in Rh-catalyzed hydrogenation reactions*

Early studies performed by Vineyard and coworkers on the substrate requirements revealed that enamides and in particular α-dehydroamino esters are excellent substrates in Rh-catalyzed asymmetric hydrogenation reactions.[33] This was attributed to the possibility of an additional coordination of the substrate to the metal center provided by the carbonyl moiety of the amide as depicted in Figure 1.8. The carbonyl moiety acts as stereo-directing group, limiting the rotational freedom of the substrate in the chiral complex. Figure 1.8 shows some of the

results obtained during this study using Rh-DiPAMP (**L3**) (3 bar H_2, 50 °C, MeOH) in the hydrogenation of differently substituted prochiral alkenes with chelating features. The enantioselectivity obtained clearly showed the superiority of α-dehydroamino ester **1.11** over enamides **1.13** and **1.14**. Nevertheless, good enantioselectivities were also obtained using α-enol acetate **1.15**. The removal of the ester or amide moiety led to a racemate when using substrate **1.16**. In this case the acid was used, which should still ensure chelation of the substrate forming a 5-member ring with the metal center.

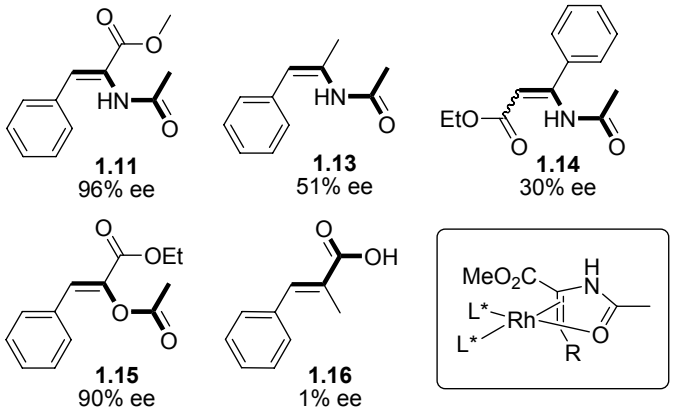
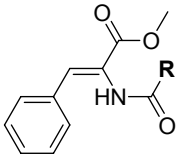


Figure 1.8 *Influence of the chelating amide or ester group*

Moreover, in an investigation conducted by Glaser and coworkers, the influence of the nature of the stereo-directing group was explored using DIOP (**L9**) as chiral ligand (1 bar H_2, rt, EtOH/benzene) in relation to the reactivity and enantioselectivity obtained, revealing the superiority of the acetamido functionality as shown in Figure 1.9.[34]



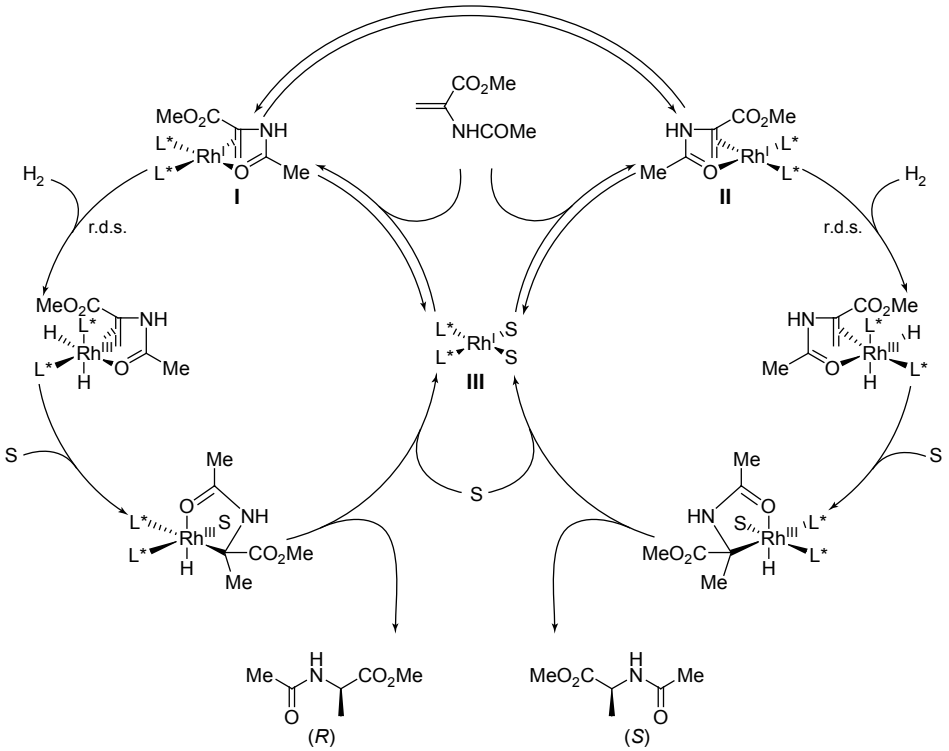
1.12: **R= Me, 69% ee**
1.17: R= H, 58% ee
1.18: R= *i*-Pr, 15% ee
1.19: R= *t*-Bu, 0% ee

Figure 1.9 *Influence of the nature of the acylamido group*

Since then, α-dehydroamino methyl esters are generally used as benchmark substrates to test the performance of newly developed catalysts,[35]

Chelation of the substrate has also been used by Halpern to formulate the now most accepted mechanism for the Rh-catalyzed asymmetric hydrogenation, a simplified version of which is depicted in Scheme 1.3.[36] The rate determining step of the reaction is the oxidative addition of H_2, which follows the coordination of the substrate to the metal center. This coordination gives rise to two diastereomeric square planar complexes **I** and **II**, which are in equilibrium with each other.

Migratory insertion of the olefin in the Rh-H bond is the step in which the chiral center is established and is followed by the reductive elimination of the product. The difference in the reaction rate of the oxidative addition of H_2 between the two diastereomeric species is the reason for the enantioselectivity observed.



Scheme 1.3 *Rh-catalyzed asymmetric hydrogenation mechanism according to Halpern (S= solvent)*

As previously mentioned, Rh-catalysts are particularly effective in the hydrogenation of functionalized alkenes possessing chelating features. On the other hand, Ru-catalysts are generally more efficient in the asymmetric hydrogenation of the C=O double bond, a transformation which is also intensively studied (*vide infra*) and is used in a number of industrial applications.[25] Increasing interest is also devoted to Ir-catalysts, which are well suited for the asymmetric hydrogenation of imines[37] and *unfunctionalized* alkenes. Excellent achievements in this field have been obtained by Pfaltz and coworkers using iridium complexes with chiral P,N ligands.[38]

1.5 Monodentate chiral phosphoramidite ligands

The results obtained using DIOP (**L9**) favored the proliferation of many *bidentate* chiral phosphorus ligands and the monodentate ligands were quickly forgotten.[39] In addition, there was a general conviction that C_2-symmetry, which characterized the majority of the bidentate ligands developed, was necessary in order to obtain high enantioselectivities. This was explained in terms of a more defined metal-complex, reduced conformational freedom and as a consequence a reduced number of possible isomeric catalyst-substrate complexes. Nevertheless, the more recent introduction of the successful ferrocene-based bidentate phosphine ligand Josiphos (**L5**, Figure 1.5)[35b] showed that the use of C_2-symmteric ligands was not *per se* essential.[40] Furthermore, most of the research was directed to the preparation of new bidentate *phosphine* ligands, as P-O and P-N based ligands were considered to be less stable and to induce lower rate acceleration during the catalytic transformation.

Between 1999 and 2000, the independent efforts of four research groups led to the introduction of new monodentate phosphines (**L10**),[41a] phosphonites (**L11**),[41b] phosphites (**L12**)[41c] and phosphoramidites (**L13**),[41d] as chiral ligands in Rh-catalyzed asymmetric hydrogenation (Figure 1.10). Especially the BINOL-based[42] phosphorus ligands **L11-13** attracted considerable attention. Since their appearance, these classes of ligands have demonstrated to be capable of excellent and sometimes unprecedented performances.[43]

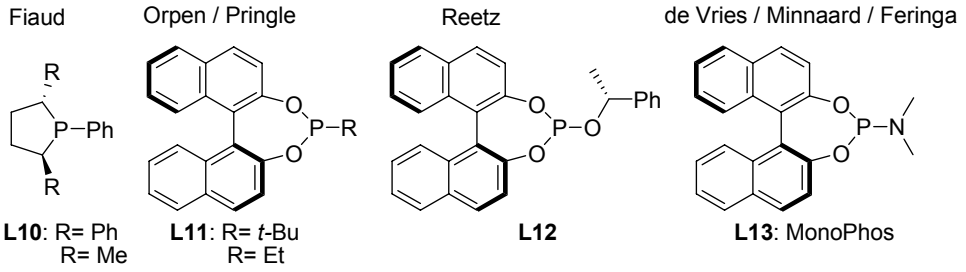


Figure 1.10 *Monodentate phosphorus-based ligands introduced in 1999-2000*

Encouraged by the excellent results obtained using MonoPhos (**L13**) in the hydrogenation of differently substituted aromatic α-dehydroamino acid derivatives (92-99% ee),[44] more BINOL-based monodentate phosphoramidite ligands[45] were developed in our laboratories (and in collaboration with DSM) and they have been successfully used in the Rh-catalyzed hydrogenation of different classes of substrates as depicted in Figure 1.11.[46]

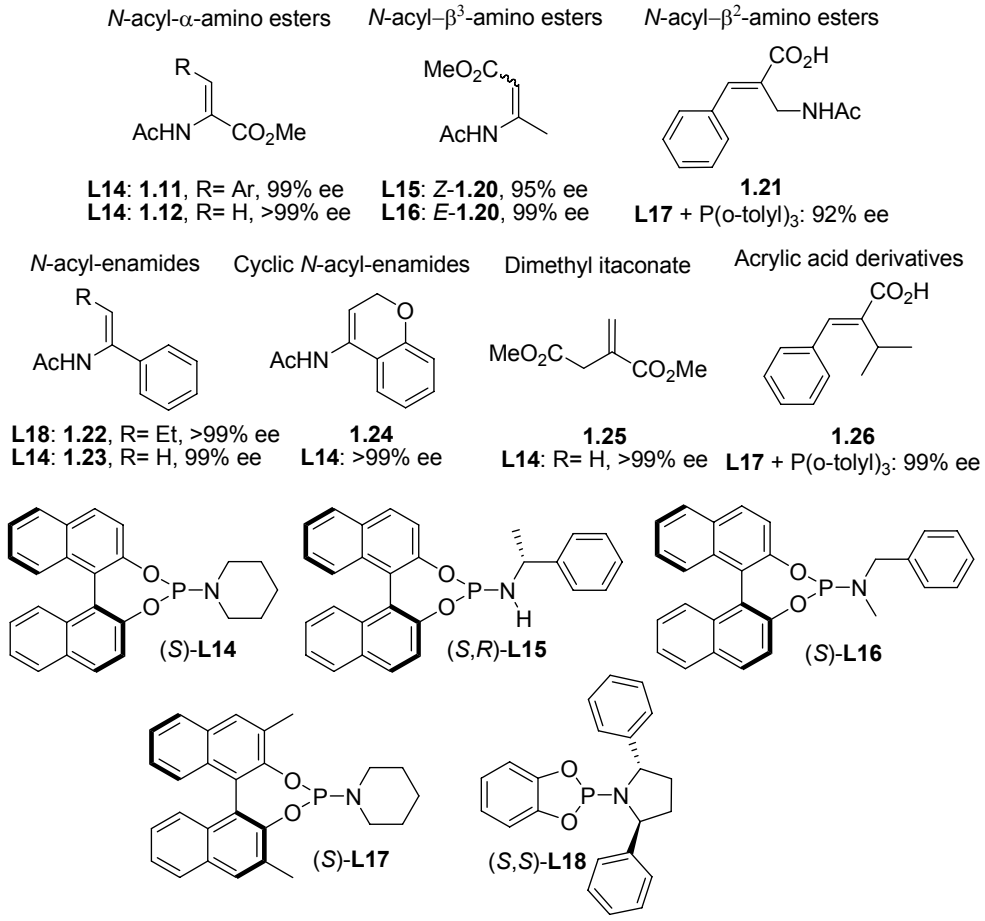


Figure 1.11 *Selected asymmetric hydrogenations using monodentate phosphoramidite ligands*

Often, the use of a differently substituted phosphoramidite ligand was necessary in order to achieve the best results in the hydrogenation of different classes of substrates. The ligand with the broadest substrate scope resulted to be PipPhos (**L14**) which provided almost perfect enantioselectivities in the hydrogenation of the benchmark substrates (Z)-2-(acetamido)cinnamic and 2-(acetamido)acrylic methyl esters (**1.11** and **1.12**), differently substituted enamides (**1.23** and **1.24**) and dimethyl itaconate (**1.25**).[47] However, very interestingly, the best results in the hydrogenation of the bulkier *N*-acyl-enamide **1.22** were achieved using the catechol-based phosphoramidite **L18**, which possesses a chiral amine but an achiral backbone.[48] Two different phosphoramidites **L15** and **L16** were, instead, necessary to achieve high enantioselectivities in the hydrogenation of the *E* and *Z* isomers of *N*-acyl-β^3-dehydroamino acids **1.20** (in CH_2Cl_2 and *i*-PrOH,

respectively).[49] For the hydrogenation of the less studied, but not less interesting, *N*-acyl-β^2-dehydroamino acids **1.21** and of the substituted acrylic acids **1.26** a completely new protocol was developed in collaboration with DSM, defined as ligand combination approach. The most successful Rh-catalyst involved the use of a mixture of the bulky monodentate phosphoramidite **L17** and the bulky achiral tri-*ortho*-tolylphosphine. In both cases, due to the lack of a chelating acetamide moiety, the use of the free acid turned out to be necessary in order to achieve high enantioselectivities. Probably in this case the acid is providing the extra coordination to the metal center.[50]

Among the classes of substrates which have not been investigated or which so far could not be hydrogenated with high enantioselectivities using monodentate phosphoramidites ligands are: (i) enol acetates (see Chapter 2); (ii) alkyl-substituted α-dehydroamino esters (see Chapter 3); (iii) tetra-substituted α-dehydroamino esters (see Chapter 3); (iv) ketones, imines and (hetero-)aromatics.

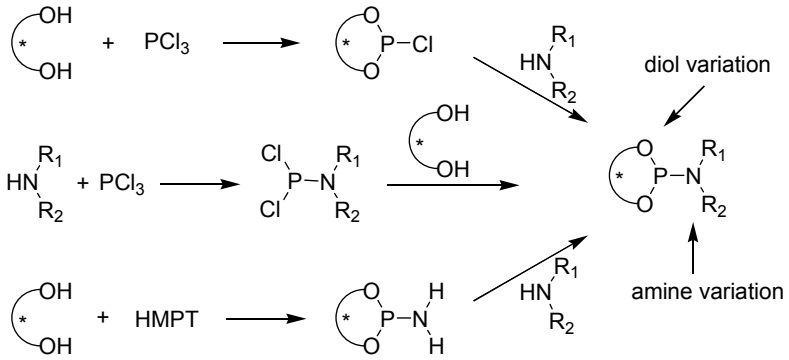
1.5.1 Approaches to ligand differentiation

There is a clear interest in applying asymmetric hydrogenation on industrial scale as demonstrated by the development of chiral ligands such as DIPAMP (**L3**) by Monsanto and DuPHOS (**L4**) by DuPont. Although homogeneous asymmetric catalysis has reached an advanced stage in the laboratory, the method of choice for the preparation of enantiopure intermediates, in the production of pharmaceuticals, is in many cases still the resolution of diastereomeric salts.[51] This is in general due to the presence of established stoichiometric synthesis protocols, the reluctance of medicinal chemists to use catalysis and last, but not least, the necessity to identify selective and appropriate catalysts.

Phosphorus-based chiral ligands are extremely versatile for asymmetric catalysis. Nevertheless, the availability of chiral bidentate phosphorus ligands is often associated with complicated or time consuming synthesis. This limits the possibility of ligand variation, which is essential in an exploratory stage for the identification of the most suitable ligand.[52] Often, a new catalytic reaction or a new substrate requires the use of a new chiral ligand and its identification or structural optimization is rarely achievable just by rational design. Therefore, the availability of large libraries of chiral catalysts and in particular of the corresponding ligands is highly desirable but, nevertheless, a serious bottleneck.[53]

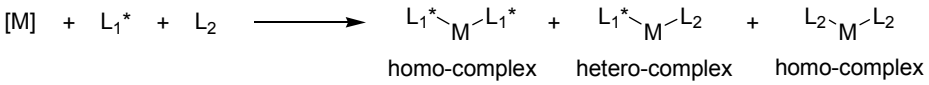
Chiral monodentate phosphoramidite ligands have the advantage of a simple and modular synthetic approach which provides a large repertoire of possible structures (Scheme 1.4). Recently, at DSM and in Groningen an *in situ* automated parallel synthesis of ligands has been achieved. This allows large libraries of ligands to be prepared. Libraries of monodentate ligands can be divided in three types: (i) libraries of homochiral monodentate ligands; (ii) use of combinations of two chiral ligands from the libraries; (iii) use of chiral and achiral ligand combinations. These approaches have been used in Rh-catalyzed asymmetric conjugated additions, Rh-

and Ir-catalyzed asymmetric hydrogenations and Ru-catalyzed asymmetric transfer hydrogenations.[52b,54]



Scheme 1.4 *Modular structure of the chiral monodentate phosphoramidites and routes to prepare these ligands*

As mentioned, the use of monodentate ligands allows the use of catalysts in which two different ligands have been mixed.[55] In this mixture one of the ligands might be achiral (Scheme 1.5). This broadens the catalyst differentiation and gives the opportunity to easily access Rh-complexes that are not symmetric (see also Chapter 3).[52b,45b] This approach, as previously mentioned, formed the basis for the successful hydrogenation of *N*-acyl-β[2]-amino acids **1.21** and substituted acrylic acids **1.26** (Figure 1.11).



Scheme 1.5 *Hetero- and homo-combination of monodentate ligands*

The search for ligand differentiation and novel chiral scaffolds to use as ligands in transition metal-catalyzed reactions has led to the recent development of approaches, such as: (i) libraries of peptides in which phosphine containing amino acids were incorporated;[56] (ii) DNA-intercalating based catalysts;[57] (iii) supramolecular bidentate phosphorus-based ligands consisting of BINOL and porphyrine moieties;[58] (iv) self-assembly of chiral monodentate ligands into bidentate systems by complementary A-T base pair analogues.[59]

In this respect, an interesting approach to ligand differentiation can be found in the late seventies works by Kaiser,[60] Whitesides[61] and their coworkers. They were the first to envision the possibility of introducing artificial cofactors into protein scaffolds, affording artificial biocatalysts with novel catalytic activities (see Chapter 5). The protein provided the chiral environment and the chemocatalyst was instead responsible for the catalytic activity observed. Beside the large number of possible protein structures already available, this approach would also allow one to take advantage of the continuous advances in molecular biology techniques for the

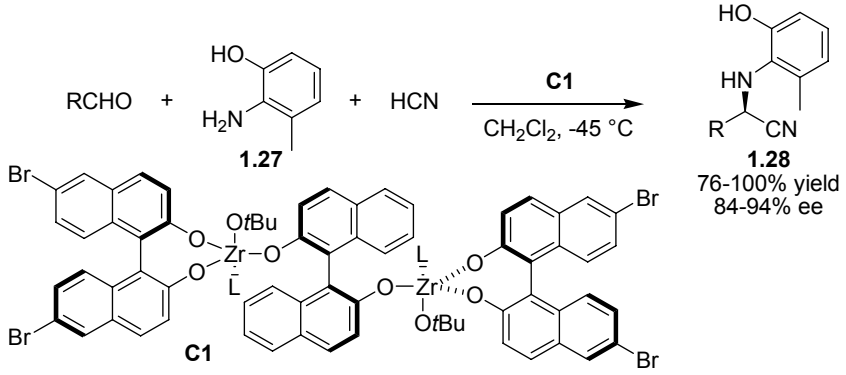
preparation of large libraries of proteins, expanding even further the combinatorial approach for the identification of suitable catalysts for all kinds of transformations.[62,63]

1.6 Enantiopure α-amino acids

Enantiopure α-amino acids are important building blocks for the preparation of pharmaceutical and agrochemical target molecules, such as peptides, proteins, peptidomimetics and other natural products.[64] Furthermore, α-amino acids are extensively used as chiral starting materials, auxiliaries, and catalysts in modern organic synthesis.[22] There are four main approaches to obtain optically active α-amino acids, namely biotechnological methods,[65] chemical synthesis using compounds from the chiral pool, resolution of racemic mixtures, and asymmetric catalysis (Figure 1.4).[66] Different catalytic asymmetric approaches to α-amino acids have been developed and they involve the formation of C-C, C-N and C-H bonds.[67]

1.6.1 Asymmetric Strecker reaction

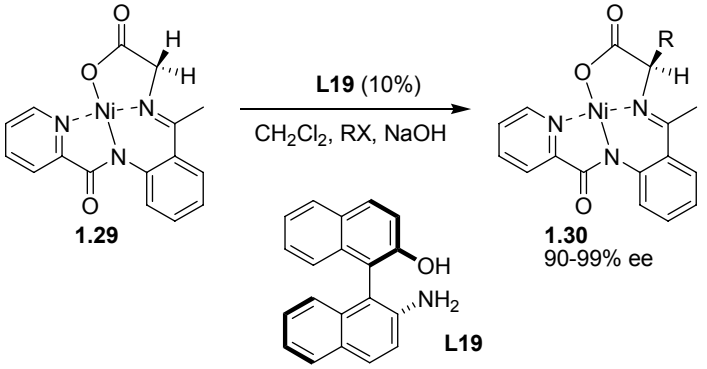
The Strecker α-amino acid synthesis, first reported in 1850, involves treatment of aldehydes with NH_3 and HCN.[68] The resulting amino nitrile is hydrolyzed to provide racemic α-amino acids. Partial hydrolysis of amino nitriles leads to racemic amino amides which resolution is achieved using amidases yielding enantiopure (L)-amino acids and (D)-amino amides.[69] Both methodologies are industrially attractive. The asymmetric version of the Strecker reaction has received increasing interest.[70] In Scheme 1.6 the asymmetric three-component reaction developed by Kobayashi and coworkers is depicted, which appears to be a promising approach with industrial potential.[71]



Scheme 1.6 *Three-component asymmetric Strecker reaction*

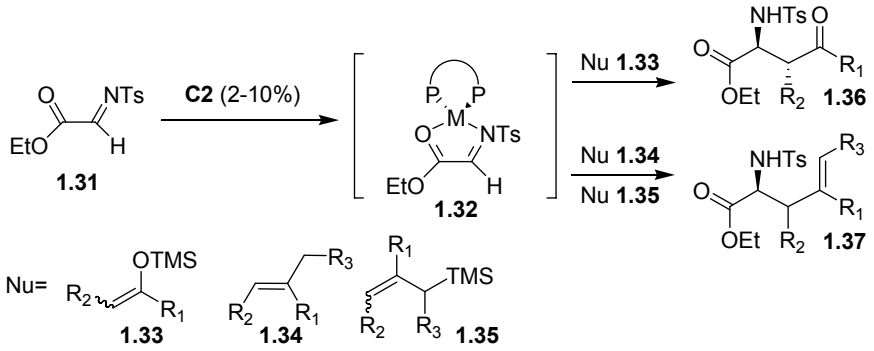
1.6.2 Asymmetric C-C bond-forming reactions

Asymmetric C-C bond-forming reactions usually involve the asymmetric alkylation of glycine equivalents that can be divided into glycine α-anion and α-cation equivalents. Among the glycine α-anion equivalents the most interesting approach recently developed is the asymmetric phase transfer-catalyzed alkylation of achiral Ni-complexes developed by Belokon and coworkers (Scheme 1.7).[72,73] The methodology is simple, efficient and has a broad scope. However, a stoichiometric amount of nickel is required and **1.29** is prepared in a separate step.



Scheme 1.7 *Asymmetric alkylation of glycine α-anion equivalent **1.29***

Lectka and coworkers, instead, presented the most general method in terms of substrate scope among the glycine α-cation approaches.[74] In this case, the reaction consists of the alkylation of carbon-based nucleophiles in a Mannich-type reaction (Scheme 1.8). The alkylation of α-imino ester **1.31** with silyl enol ethers **1.33**, alkenes **1.34** and allylsilanes **1.35** was achieved using (*R*)-tol-Binap / $CuClO_4$ complex **C2** reaching enantioselectivities up to 99% ee.

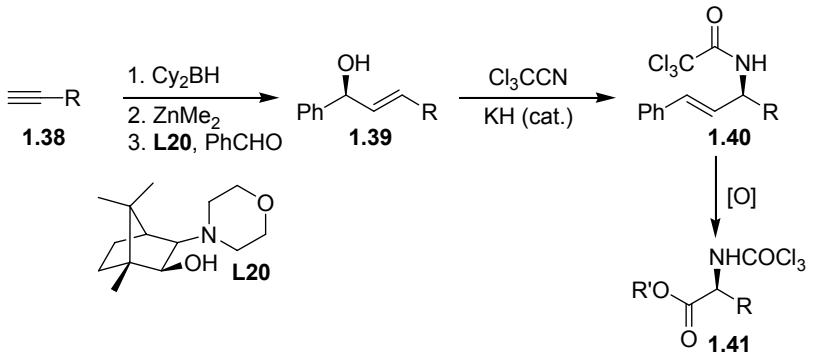


Scheme 1.8 *Asymmetric alkylation of glycine α-cation equivalent **1.31***

1.6.3 Asymmetric C-N bond-forming reactions

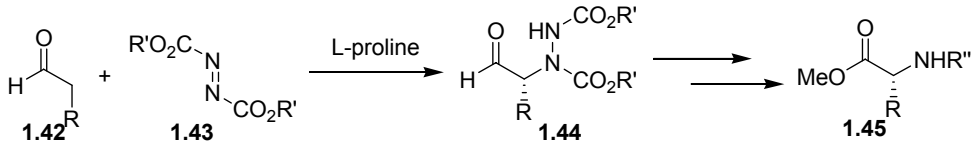
The preparation of α-amino acids by the introduction of the NH_2 moiety using amines is based on S_N2 substitutions on chiral substrates. Versatile intermediates are chiral epoxides, α-halo or α-hydroxy carboxylates.[75]

Recently, Walsh and coworkers presented a highly enantioselective synthesis yielding protected α-amino acids starting from terminal alkynes **1.38** (Scheme 1.9).[76] Chiral allylic alcohols **1.39** were obtained via asymmetric vinylation of benzaldehyde in the presence of **L20** in high enantioselectivities (88-97% ee) and converted into allyl amines **1.40** by Overman's [3,3]-sigmatropic rearrangement. Oxidative cleavage of amines **1.40** provided the α-amino acids derivatives **1.41** without loss in enantioselectivity. However, the methodology is not atom efficient.



Scheme 1.9 *Nucleophilic amination of chiral allylic alcohols **1.39***

More interesting is the methodology independently developed by Jørgensen and List and their coworkers.[77] In their approach, L-proline catalyzed the asymmetric α-amination of aldehydes **1.42** with azodicarboxylates **1.43**, as nitrogen source. The adducts **1.44,** obtained with high enantioselectivies (89-97% ee),can be converted into α-amino acids **1.45** upon oxidation (Scheme 1.10).



Scheme 1.10 *Asymmetric α-amination of aldehydes **1.42***

1.6.4 Asymmetric hydrogenation

Although significant improvements have been achieved using the catalytic asymmetric methodologies briefly described here, asymmetric hydrogenation is still the only catalytic asymmetric method for the preparation of α-amino acid

derivatives on an industrial scale. However, considering that α-dehydroamino acid derivatives are excellent substrates for Rh-catalyzed hydrogenation reactions, the efficiency and simplicity of the methodology and the plethora of chiral ligands developed, it is quite surprising that hydrogenation does not have a more prominent role at a production level. In most literature reports only (Z)-2-(acetamido)cinnamic (**1.11**) and 2-(acetamido)acrylic (**1.12**) methyl ester are used to test catalyst efficiency and not because of real interest in the products. The limited development of efficient synthetic pathways which would allow the accessibility of a wider range of differently substituted α-dehydroamino acid derivatives and an easy removal of the amine stereo-directing group at a later stage might be among the possible reasons (see Chapter 3).[78] This limits the substrate scope and makes the use of asymmetric hydrogenation less appealing as a practical tool for the preparation of α-amino acids also on large scale. Nowadays, the difficulties encountered in the preparation of α-amino acids using asymmetric hydrogenation are reported to be the synthesis of the olefinic precursors, their purity (also isomeric, *E*/*Z* mixtures) and the necessary and sometimes troublesome removal of the amine protection.[25a]

1.7 Chiral secondary alcohols

Chiral alcohols are important building blocks for the preparation of not only compounds of pharmaceutical interest but also for fragrances and agricultural chemicals and specialty materials (Figure 1.12).[24,25]

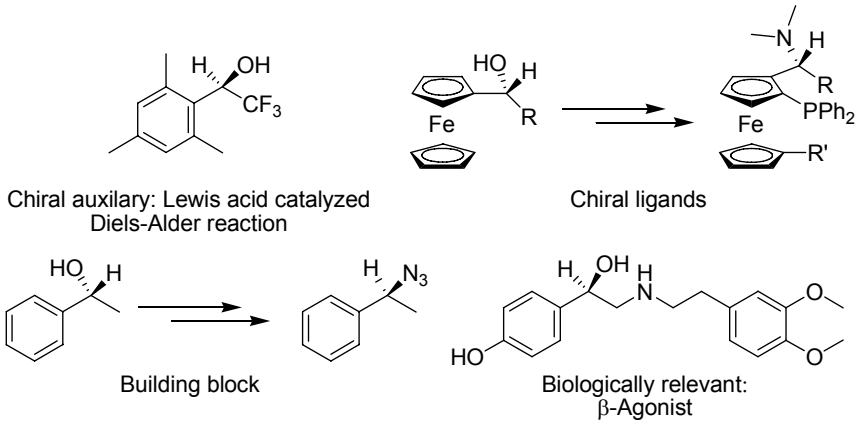


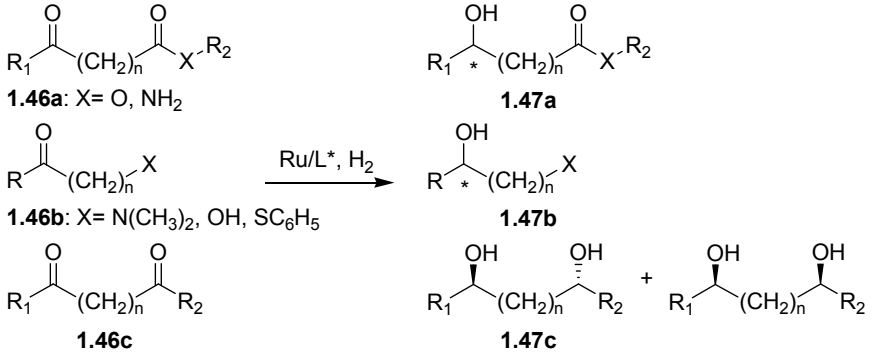
Figure 1.12 *Examples of applications of chiral alcohols*

Due to the abundance of ketones available, an obvious and practical way of obtaining chiral alcohols is by reduction of ketones. For more than fifty years, the selective reduction of carbonyl compounds to alcohols has mainly relied on the use of the hydrides of boron, aluminum and silicon. Modification of compounds such as

$LiAlH_4$, AlH_3, $NaBH_4$ and BH_3·THF with chiral ligands such as 1,2 diols, 1,2 amino alcohols and 1,2 diamines allowed the achievement of stereocontrol in the reduction of ketones.[79] However, for technical and economic reasons, particularly for large-scale reactions, catalyzed homogeneous hydrogenation is obviously more desirable than stoichiometric hydride reduction.

1.7.1 Metal-catalyzed homogeneous hydrogenation of ketones

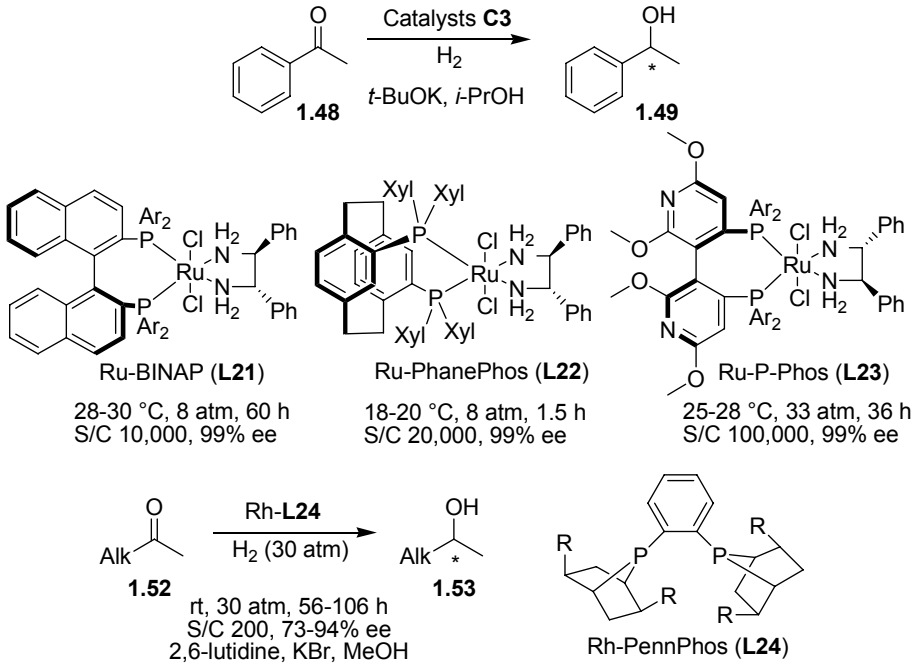
Neutral or ionic transition metal complexes, with bidentate chiral phosphines as ligands and ruthenium or rhodium as metals, have been successfully employed as catalysts in the asymmetric hydrogenation of functionalized ketones **1.46a-c** (Scheme 1.11).[80] The most versatile catalytic system is still Ru-BINAP (**L1**) due to the large variety of substrates that can be effectively hydrogenated.



Scheme 1.11 *Asymmetric hydrogenation of functionalized ketones **1.46a-c***

The successful asymmetric hydrogenation of unfunctionalized ketones was more problematic until the introduction of mixed ligand complexes such as $RuCl_2$(diphosphine)(1,2-diamine) **C3** in combination with an inorganic base.[11] The best catalytic systems for the enantioselective hydrogenation of aromatic ketones **1.48** have ruthenium as the metal, Xylyl-BINAP (**L21**),[81a] Xylyl-PHANEPHOS (**L22**)[81b] and P-Phos (**L23**)[81c] as ligands, dpen (**1.50**) and daipen (**1.51**) as amines, KOH and *t*-BuOK as bases (Scheme 1.12).

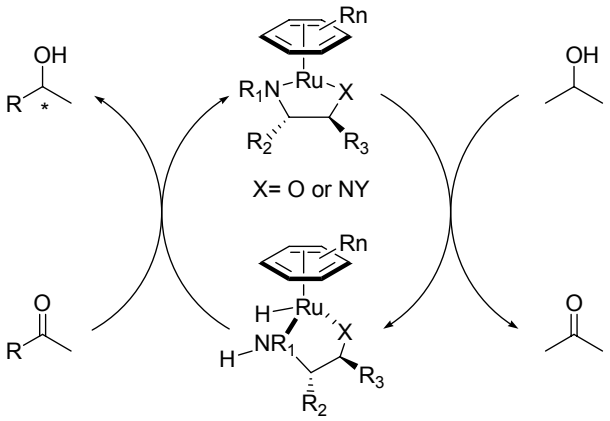
The enantioselective hydrogenation of dialkyl ketones turned out to be less efficient. Good results have been achieved using Rh-PennPhos (**L24**) in combination with 2,6-lutidine and KBr as additives (Scheme 1.12).[82] However, the system turned out to be less reactive, as longer reaction times were necessary to reach full conversion. Moreover, it should be noted that all these systems are highly efficient only in the presence of a strong base. These conditions limit somewhat the scope of the reaction being not suitable for configurationally labile ketones. Further research allowed Noyori and coworkers to obtain the more robust pre-catalyst *trans*-RuH(η^1-BH_4)(BINAP)(DPEN), which was able to perform the reaction under base-free conditions.[83]



Scheme 1.12 *Hydrogenation of simple ketones **1.48** and **1.52***

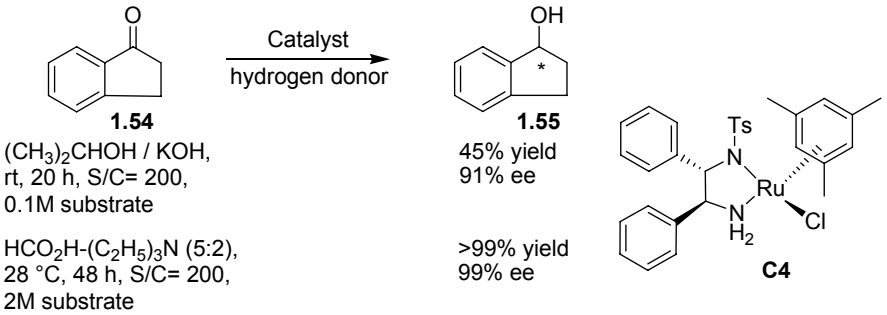
1.7.2 Transfer hydrogenation

Reduction of ketones can also be effected *via* catalytic hydrogen transfer reactions. In this case, the hydrogen is transferred from suitable donor molecules such as 2-propanol. The metal that is mostly used is ruthenium, but also rhodium and iridium can be applied. In general, chiral 1,2 amino alcohols and 1,2 di-amines are used as ligands (Scheme 1.13).[35c,80a-b,84] Bases are often crucial co-catalysts. Although the methodology seems to be advantageous, as no pressure equipment and hydrogen gas are required, the reaction is reversible and therefore it is difficult to reach full conversion with concomitant high enantioselectivity.



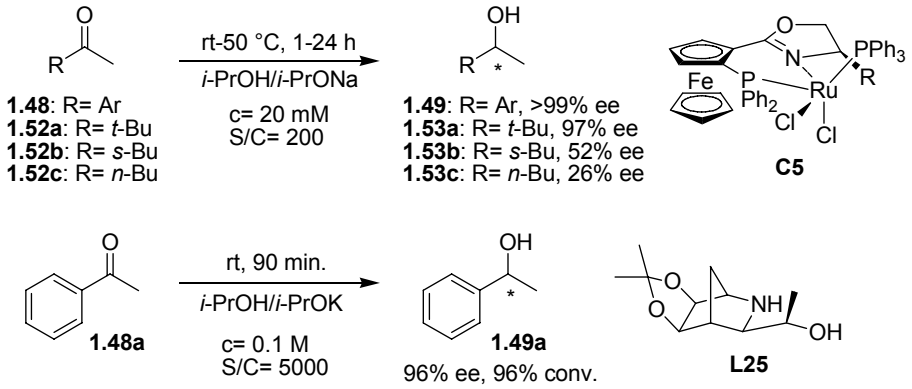
Scheme 1.13 *Transfer hydrogenation pathway*

A partial solution to this problem has been achieved by using diluted solutions (usually 0.1M in substrate). Moreover, if ammonium formate is used instead of 2-propanol the co-product is CO_2, which escapes from the reaction mixture and eliminates the reversibility problem. Nevertheless, not all the catalysts tolerate the use of formic acid. In Scheme 1.14, an example of the use of both hydrogen donors with the catalyst developed by Noyori and coworkers is shown.[85]



Scheme 1.14 *Hydrogen donors in transfer hydrogenation reactions*

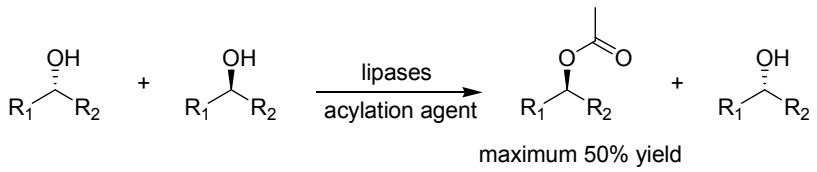
Very good enantioselectivities have been achieved for a variety of aryl methyl ketones (>99% ee) using Ru(II)-oxazolinylferrocenylphosphine complex **C5**.[86] Improvements have been made also on the activity using the amino alcohol based ligands **L25** developed by Andersson and coworkers.[87] Nevertheless, also in this field the reduction of di-alkyl ketones **1.52** remains problematic. Incomplete conversions and moderate to good enantioselectivities have been achieved (26%-99% ee) depending on the substrate (Scheme 1.15).[86]



Scheme 1.15 *Recent advances in transfer hydrogenation of simple ketones*

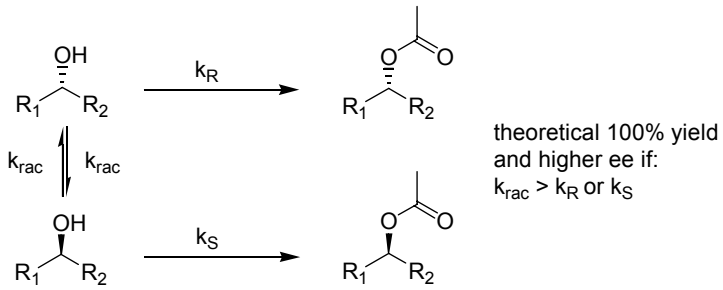
1.7.3 Chiral alcohols from biocatalytic processes

Using biocatalysis, chiral alcohols can be obtained by kinetic resolution of racemates or by direct synthesis from ketones. Nowadays, kinetic resolution by enzyme catalysis is a well-known and applied process. Many examples are present in the literature of lipase-catalyzed hydrolysis of esters and acylation of alcohols (Scheme 1.16).[88] A drawback is that the resolution of racemates using enzymatic acylation affords a maximum of 50% yield of the desired enantiomer. As also the reverse reaction can take place, it is important to remove water formed during the reaction or to use an excess of acylating agent. Isopropenyl acetate is often used, as the alcohol formed tautomerizes and cannot participate in the reverse reaction.



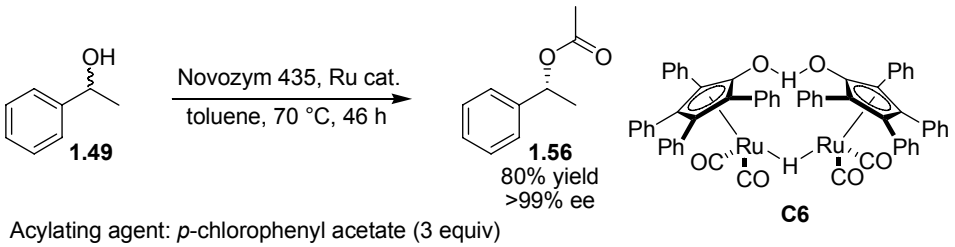
Scheme 1.16 *Enzyme catalyzed resolution of secondary alcohols*

As an alternative, a dynamic kinetic resolution (DKR) approach based on the combination of resolution operated by lipases and racemization operated by ruthenium catalysts allows a theoretical 100% yield (Scheme 1.17).[89]



Scheme 1.17 *Dynamic kinetic resolution of secondary alcohols*

In order to achieve a reasonable rate of racemization it is essential to add some ketone, such as acetophenone (**1.48**), as the racemization proceeds through a dehydrogenation-hydrogenation sequence. The system developed by Bäckvall and coworkers is depicted in Scheme 1.18. The acylating agent adopted in this case is *p*-chlorophenyl acetate as the resulting alcohol does not retard the Ru-catalyzed racemization.[90]



Acylating agent: *p*-chlorophenyl acetate (3 equiv)

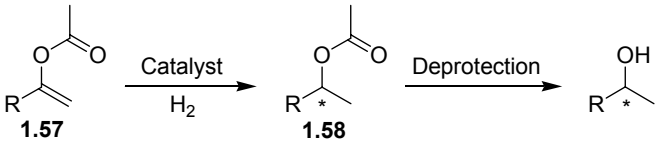
Scheme 1.18 *Example of dynamic kinetic resolution*

Remarkably, compared to other methods mentioned, Bäckvall reported that in this way it was possible to obtain 2-octanol in good yield (80%) and high enantiomeric excess (97%).[90] The methodology is indeed interesting, although reaction times are still rather long and the reactions do not go to completion. In particular, the presence of *p*-chlorophenol in the reaction mixture is not desirable due to its toxicity.[91]

More recently, increasing attention has been devoted to the direct reduction of ketones using dehydrogenases and reductases in the presence of NADH and NADPH as cofactors.[92]

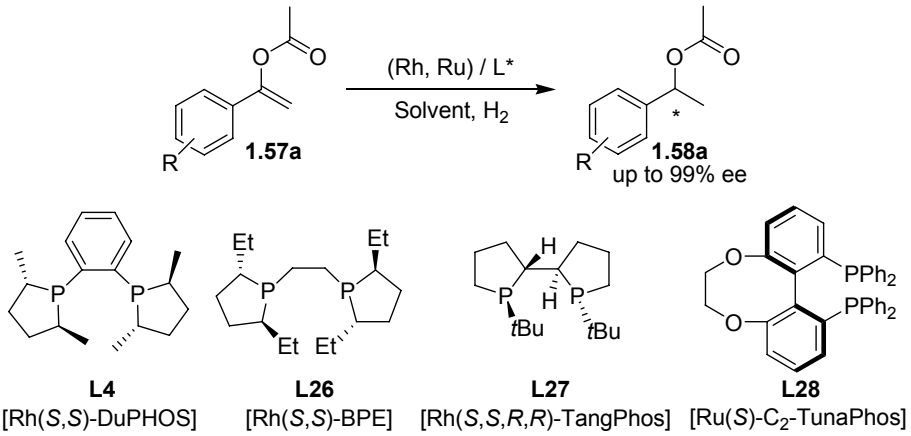
1.7.4 Chiral alcohols from Rh-catalyzed hydrogenation of enol acetates

The asymmetric hydrogenation of enol acetates (**1.57**, Scheme 1.19) gives access to chiral esters (**1.58**), which makes this method an interesting alternative to the enantioselective hydrogenation of prochiral ketones for the preparation of chiral alcohols.



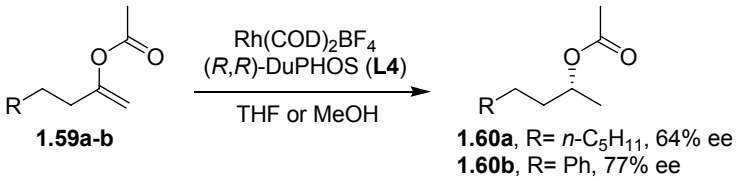
Scheme 1.19 *Alternative pathway for the preparation of chiral alcohols*

However, only few successful ligands have been reported for this transformation and modest enantioselectivities (up to 81% ee) were reached[93] until Burk reported the hydrogenation of simple aromatic enol acetates **1.57a** (up to 90% ee) using DuPHOS (**L4**) and BPE (**L26**, Scheme 1.20).[94] In one of the most recent examples[95] (Scheme 1.20), enantioselectivities up to 99% were achieved using Rh-TangPhos (**L27**) for the hydrogenation of aromatic acyclic enol acetates **1.57a**.[95b] Catalysts such as Ru-TunaPhos (**L28**)[95c] and Rh-PennPhos (**L24**)[95f] were found to be more efficient for cyclic enol acetates **1.57b** with enantioselectivities up to 99% ee.



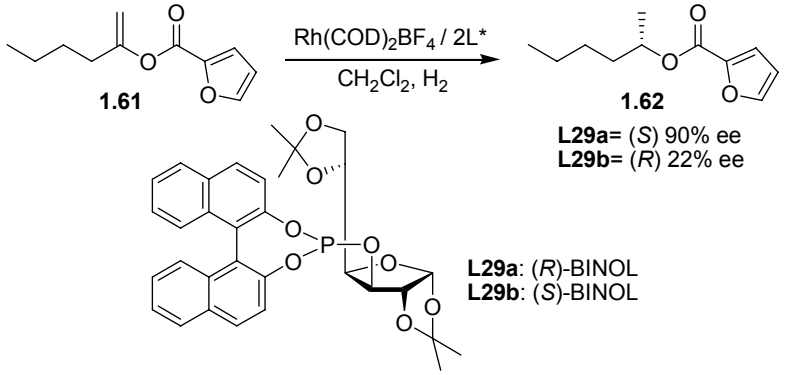
Scheme 1.20 *Acyclic aromatic vinyl acetates **1.57a** as hydrogenation substrates*

Nevertheless, the substrate scope is limited to aryl enol acetates. Boaz reported the only example of the use of a chiral bidentate phosphine ligand (**L4**) in the Rh-catalyzed asymmetric hydrogenation of aliphatic enol acetates **1.59** (Scheme 1.21).[96] The enantioselectivities are modest compared to what has been achieved with 2-aryl enol acetates **1.57a**, confirming the fact that aliphatic substrates are more difficult to hydrogenate with high enantioselectivities, as was observed when using other methodologies described in this chapter.



Scheme 1.21 *Hydrogenation of enol acetates bearing 1-alkyl substituents (**1.59**) using DuPHOS (**L4**).*

Recently, Reetz and coworkers reported the only successful use of monodentate ligands.[97] Enantioselectivities up to 90% ee were found at room temperature (94% ee at -20 °C) for the alkenyl carboxylate **1.61**, using the carbohydrate-derived Rh-monophosphite catalyst **L29a**.



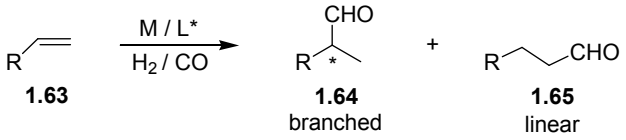
Scheme 1.22 *Hydrogenation of alkyl vinyl carboxylate **1.61** with monodentate phosphite ligands **L29a-b**.*

Although Ru-catalyzed asymmetric ketone hydrogenation seems to be very efficient (*vide supra*) and ketones are widely available, the use of additives and the limited substrate scope suggest that the identification of new catalytic routes is indeed desirable. In this respect, the Rh-catalyzed asymmetric hydrogenation of enol acetates could provide a very useful contribution and should be further investigated, especially taking into account the simplicity and mildness of the reaction conditions.

1.8 Rh-catalyzed asymmetric hydroformylation

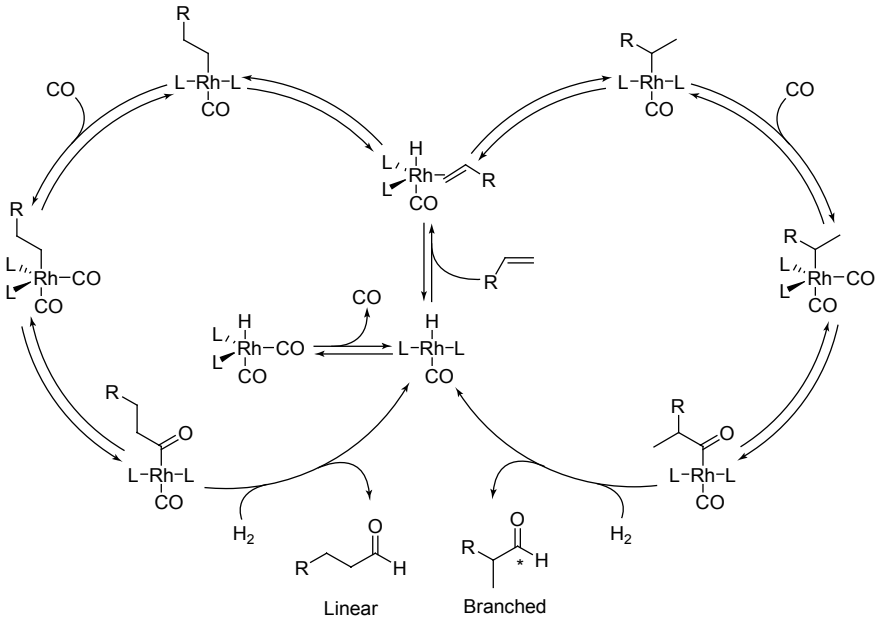
The hydroformylation reaction involves the formation of a new C-C bond and at the same time the introduction of a new functional group, yielding branched **1.64** and linear **1.65** products starting from alkenes **1.63** and H_2/CO gas mixtures (Scheme 1.23). After its discovery by Roelen in 1938,[98] studies on the hydroformylation reaction have been characterized by the use of cobalt based catalysts, until

Wilkinson and coworkers introduced the first Rh-phosphine complexes in the late 1960s.[99] Nowadays, the majority of the hydroformylation catalysts used are based on rhodium, despite various reports on the use of other metals such as Pt, Pd, Ir, Ru and Fe.[100] Rhodium catalysts are characterized by higher activities and regioselectivities, besides being active under mild conditions without the use of additives or promoters and showing negligible presence of hydrogenation or isomerization products.



Scheme 1.23 *The products of the hydroformylation reaction are branched and linear aldehydes*

The reaction mechanism depicted in Scheme 1.24 is the generally accepted dissociative mechanism proposed by Wilkinson and Brown.[101] According to this mechanism, the catalytic cycle starts with the dissociation of a CO ligand from the catalyst resting state, a dicarbonyl rhodium hydride complex. At this point, alkene association and migratory insertion of the alkene in the Rh-H bond are responsible for the formation of either the linear or branched alkyl rhodium intermediate which will yield the two different branched and linear products.



Scheme 1.24 *Rh-catalyzed hydroformylation mechanism*

The hydroformylation reaction is one of the most important industrial processes involving the use of homogeneous metal catalysts. In this respect, many studies have been devoted to the development of highly regioselective catalysts for the preparation of the industrially more interesting linear aldehydes **1.65** from 1-alkenes or the isomerization of internal alkenes.[102]

During the reaction a new stereogenic carbon is obtained for the branched product **1.64** making the metal-catalyzed *asymmetric* hydroformylation reaction a very attractive tool for the preparation of enantiomerically enriched aldehydes, which can be used as building blocks in the synthesis of pharmaceuticals or biologically active compounds (Figure 1.13).[103]

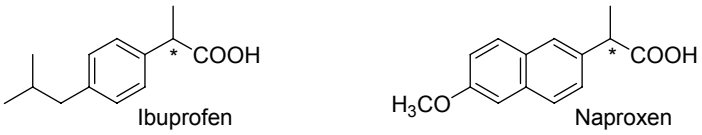
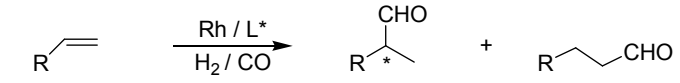


Figure 1.13 *Anti-inflammatory arylpropionic acids as targets for asymmetric hydroformylation reactions*

The catalytic enantioselective version of the hydroformylation reaction appeared in the early 1970s with the introduction of the first Rh(I)-chiral phosphine complexes.[104] The field is still in a developing stage and the alkenes depicted in Scheme 1.25 are the most common benchmark substrates **1.63a-c** used in order to identify new catalysts and compare their performances.[105]



1.63a: R= Ph
1.63b: R= OAc
1.63c: R= CH_2CN

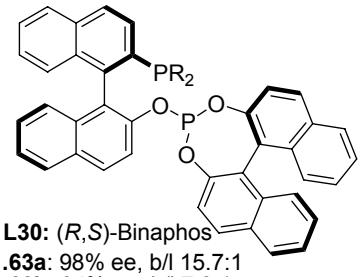
1.64a-c branched

1.65a-c linear

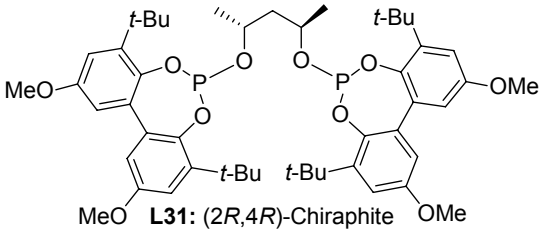
Scheme 1.25 *Benchmark substrates **1.63a-c** used in asymmetric Rh-catalyzed hydroformylation reactions.*

Although Rh-catalyzed asymmetric hydroformylation is developing slower than Rh-catalyzed asymmetric hydrogenation, excellent enantioselectivities and reasonable activities have been achieved with Rh-catalysts using diphosphite (up to 93% ee)[106] or phosphite-phosphine (up to 98% ee)[107] ligands. Recently, comparable results in terms of activity, regioselectivity and enantioselectivity have been achieved using Rh-diphosphine complexes (up to 96% ee) such as DuPHOS (**L4**) and BPE (**L26**),[108] already well-known ligands in Rh-catalyzed asymmetric hydrogenation reactions. The structures of the most successful bidentate phosphorus-based ligands **L30-36** tested in the Rh-catalyzed hydroformylation of standard substrates **1.63a-c** are depicted in Figure 1.14. Despite the good results obtained with a number of systems, Rh-Binaphos (**L30**) still remains the most versatile catalyst developed due to the wide substrate scope[105b] although the

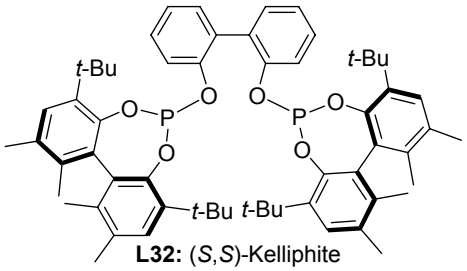
reasons of its efficiency are still not completely understood.[109] However, the high ligand-to-rhodium ratio (4:1) and syngas pressures (up to 100 bar) required using Binaphos (**L30**) and its difficult preparation make it desirable to find alternative efficient and versatile catalytic systems.[107]



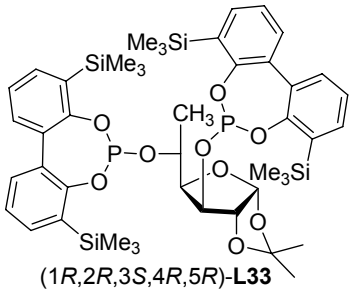
L30: (*R*,*S*)-Binaphos
1.63a: 98% ee, b/l 15.7:1
1.63b: 94% ee, b/l 7.3:1
1.63c: 66% ee, b/l 2.6:1



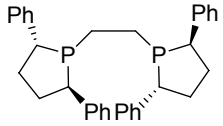
L31: (2*R*,4*R*)-Chiraphite
1.63a: 90% ee, b/l 49:1



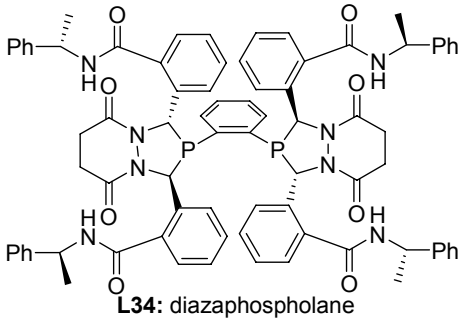
L32: (*S*,*S*)-Kelliphite
1.63c: 75% ee, b/l 15.1 :1



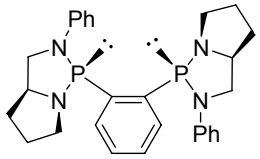
(1*R*,2*R*,3*S*,4*R*,5*R*)-**L33**
1.63a: 93% ee, b/l 82:1



L35: (*R*,*R*)-Ph-bpe
1.63a: 94% ee, b/l 45:1
1.63b: 82% ee, b/l 340:1
1.63c: 90% ee, b/l 7.1 :1



L34: diazaphospholane
1.63a: 82% ee, b/l 6.6:1
1.63b: 96% ee, b/l 37:1
1.63c: 87% ee, b/l 4.1 :1



L36: (*S*,*S*)-Esphos
1.63b: 89% ee, b/l 17.2:1

Figure 1.14 *Best results obtained in Rh-catalyzed hydroformylation in terms of enantioselectivity using bidentate phosphorus based ligands* ***L30-36***

Once more, the field is dominated by the use of bidentate phosphorus-based ligands, which proved their superior efficiency in terms of regioselectivity and

especially enantioselectivity compared to the results obtained during the sporadic studies involving the use of monodentate ligands (see Chapter 4).[105b]

1.9 Aim of this thesis

As previously mentioned, the monodentate phosphoramidite ligands developed in our laboratories are today an established and successful class of chiral ligands in Rh-catalyzed asymmetric hydrogenation.

In order to expand the scope of their use, it was decided to challenge their efficiency as ligands in the Rh-catalyzed asymmetric hydrogenation of more difficult substrates. In this respect, *Chapter 2* will describe the successful use of monodentate phosphoramidite ligands in the Rh-catalyzed asymmetric hydrogenation of enol acetates **1.57** and of newly developed enol carbamates as precursors for the preparation of chiral alcohols.

In *Chapter 3* the efficient preparation and Rh-catalyzed asymmetric hydrogenation of *N*-formyl α-dehydroamino esters will be described. The synthetic procedure applied and the use of the *N*-formyl protection allow to obtain a wider range of α-amino acid derivatives under mild reaction conditions and offers the advantage of an easy and mild removal of the formyl protecting group. Moreover, their use allowed addressing issues like: alkyl-substitution, tetra-substitution and efficiency in the hydrogenation of *E* and *Z* α-dehydroamino ester isomers.

Chapter 4 describes the results of a preliminary study conducted in order to assess the feasibility of the use of monodentate phosphoramidites as chiral ligands in the Rh-catalyzed asymmetric hydroformylation of styrene (**1.63a**) and vinyl acetate (**1.63b**) as benchmark substrates.

Finally, the successful preparation of a hybrid enzyme-bound rhodium catalyst with a single phosphorus donor ligand, which was tested in hydrogenation and hydroformylation reactions, will be described in *Chapter 5*. The use of proteins as chiral scaffolds appears an appealing and unique opportunity for broadening even further the variety of possible catalysts available and for performing metal-based homogeneous catalysis in water.[110]

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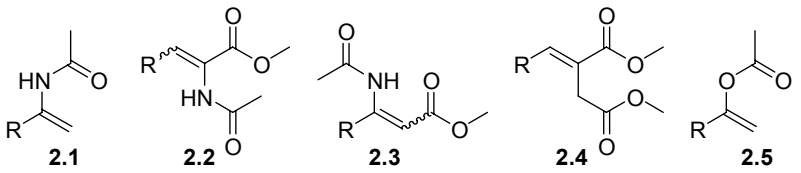
Chapter 2
Enantioselective Rh-Catalyzed Hydrogenation of Enol Acetates and Enol Carbamates with Monodentate Phosphoramidites

Monodentate phosphoramidites, in particular PipPhos and its octahydro analogue, are excellent ligands for the rhodium-catalyzed asymmetric hydrogenation of aromatic enol acetates, enol carbamates and 2-dienol carbamates in up to 98% ee. These latter substrates were hydrogenated selectively to the carbamates of the allyl alcohol.

Part of this Chapter has been published: Panella, L.; Feringa, B. L.; de Vries, J. G.; Minnaard, A. J. *Org. Lett.* **2005**, *7*, 4177.

2.1 Introduction

Homogeneous asymmetric hydrogenation of prochiral olefins has proven to be one of the most powerful tools in asymmetric catalysis.[1,2] In most of the cases, only solvent, substrate, hydrogen and a small amount of catalyst are needed, making this technology clean and atom-efficient.[3] In particular, the combination of rhodium and phosphorus based chiral ligands has proven to be essential for the development and fast advances in homogeneous asymmetric hydrogenation.[4] In Scheme 2.1 some representative classes of olefins used as substrates are depicted. In many cases, the reaction is performed at room temperature using only moderate hydrogen pressure. Neither bases, nor acids are usually required, providing mild operational conditions that allow the presence of sensitive functionalities.



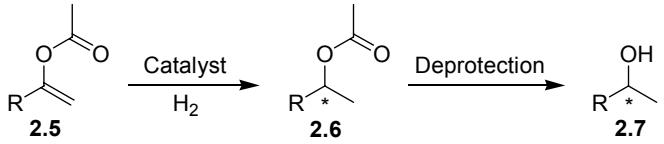
Scheme 2.1 *Classes of prochiral olefins used in Rh-catalyzed asymmetric hydrogenation*

Chiral alcohols are important building blocks and development of enantioselective procedures that allows their preparation with a minimum amount of waste or additives and with a large substrate scope is highly desirable.

As discussed in Chapter 1, an important contribution to the homogeneous asymmetric hydrogenation field is provided by the ruthenium-catalyzed asymmetric hydrogenation of prochiral ketones for the preparation of chiral alcohols. Ru-catalysts are based on bis-phosphine and diamine ligands. Variation of the bis-phosphine is cumbersome because of lengthy syntheses and extensive purifications. Therefore, in order to be able to rapidly find a higher enantioselective catalyst for a new substrate it would be highly advantageous to have a catalyst based on simple monodentate ligands, such as phosphoramidites, phosphites or phosphonites that can be easily made even using a robotic system.[5]

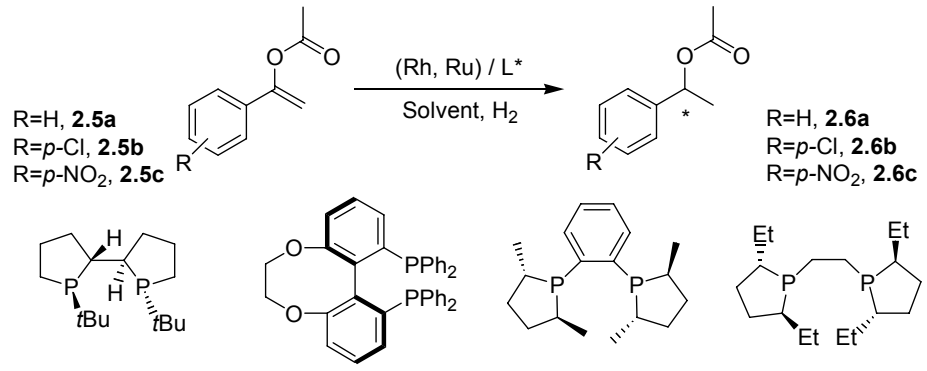
2.2 Hydrogenation of enol acetates

The asymmetric hydrogenation of enol acetates **2.5** gives access to chiral esters **2.6**, which makes this method an interesting alternative to the enantioselective hydrogenation of prochiral ketones for the preparation of chiral alcohols **2.7** (Scheme 2.2).



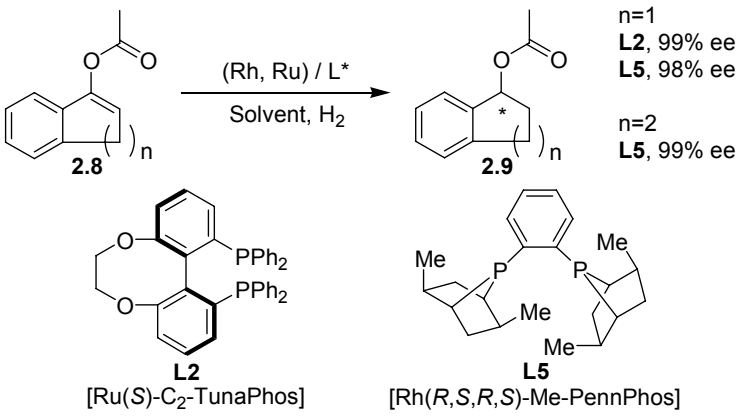
Scheme 2.2 *Alternative pathway for the preparation of chiral alcohols*

Very few ligands have been reported to be successful for this transformation in spite of the structural similarity with enamides **2.1**, the majority of which can be hydrogenated with high enantioselectivities.[4] Modest enantioselectivities (up to 81% ee) were reached[6] until Burk reported the hydrogenation of simple enol acetates such as **2.5a** (89% ee) and **2.5c** (90% ee) using DuPHOS (**L3**) and BPE (**L4**) ligands (Scheme 2.3).[7] In one of the most recent examples[8] (Scheme 2.3), enantioselectivities up to 99% ee were achieved using Rh-TangPhos (**L1**) for the hydrogenation of aromatic acyclic enol acetates.[8b]



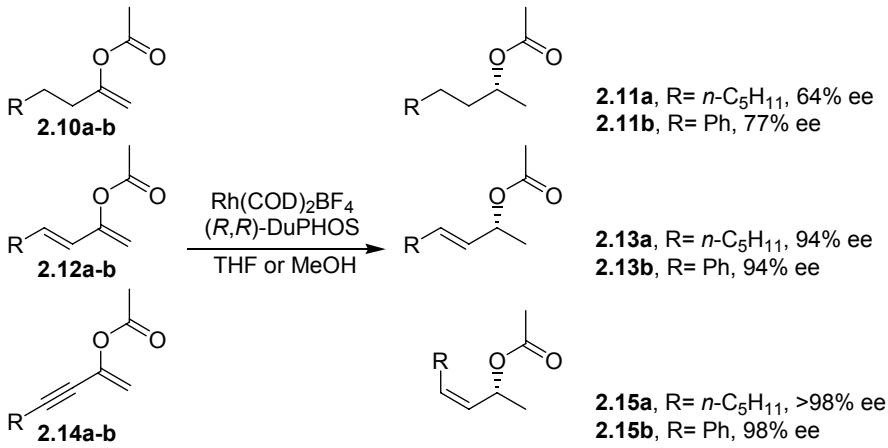
Scheme 2.3 *Acyclic aromatic vinyl acetates **2.5a-c** as hydrogenation substrates*

Catalysts such as Ru-TunaPhos (**L2**)[8c] and Rh-PennPhos (**L5**)[8f] were found to be more efficient for cyclic enol acetates such as **2.8** (up to 99% ee). The most significant results are depicted in Scheme 2.4.



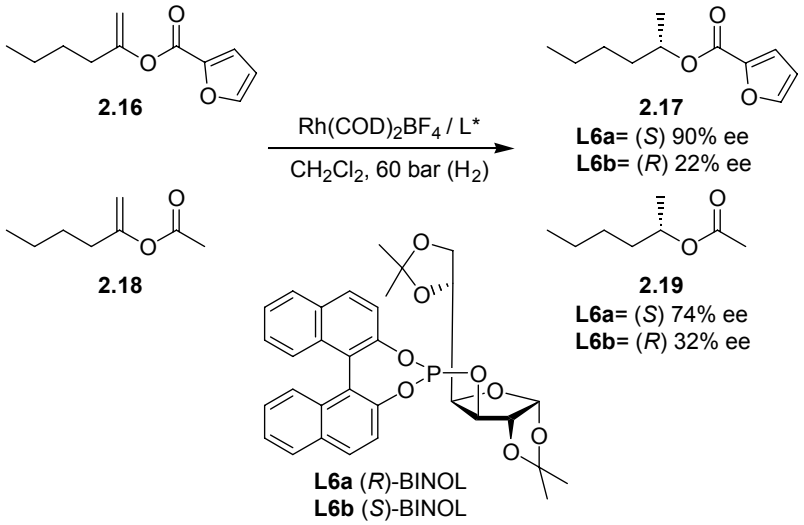
Scheme 2.4 *Cyclic aromatic vinyl acetates (**2.8**) as hydrogenation substrates*

In most cases, reaction times between 12 and 14 h have been reported and the substrate scope is limited to aryl enol acetates. Boaz reported the only example of the use of a chiral bidentate phosphine ligand in the Rh-catalyzed asymmetric hydrogenation of aliphatic enol acetates.[9] Using bidentate DuPHOS (**L3**), 64% ee and 77% ee were obtained in the hydrogenation of simple aliphatic enol acetates **2.10a-b** (Scheme 2.5). Moreover, in this case reaction times of 2 h using 2 bar H_2 were reported. The enantioselectivities are modest compared to what has been achieved with 2-aryl enol acetates **2.5a** and **2.5c**, confirming the fact that aliphatic substrates are more difficult to hydrogenate with high enantioselectivities, as observed when using other methodologies (see Chapter 1). Much higher enantioselectivities where instead obtained in the hydrogenation of 1-alkenyl **2.12a-b** and 1-alkynyl enol acetates **2.14a-b** (Scheme 2.5). Whether this increase in enantioselectivity was due to reduced steric hindrance or coordination of the extra unsaturated moiety (alkene, acetylene) to the metal center was not clarified.



Scheme 2.5 *Hydrogenation of enol acetates bearing 1-alkyl (**2.10a-b**), 1-alkenyl (**2.12a-b**) or 1-alkynyl (**2.14a-b**) substituents using DuPHOS (**L3**).*

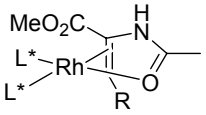
Reetz and coworkers recently reported the only example so far of a successful use of monodentate ligands (Scheme 2.6).[10] Enantioselectivities up to 90% ee were found at room temperature (94% ee at -20 °C) for the more difficult alkenyl carboxylate **2.16**, using a carbohydrate-derived Rh-monophosphite catalyst, containing two sources of chirality (**L6a-b**).



Scheme 2.6 *Hydrogenation of alkyl vinyl carboxylates with monodentate phosphite ligands*

The presence of the furanyl group in **2.16** seemed to be essential, as lower enantioselectivity was obtained with the more common aliphatic vinyl acetate **2.18**

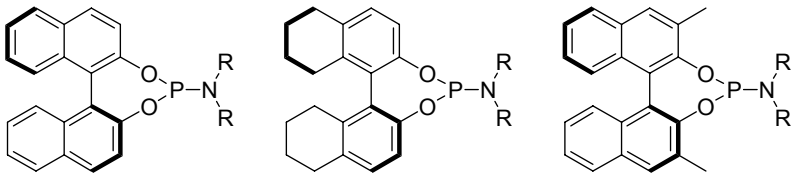
(Scheme 2.6). Catalyst **L6a** appeared to be quite stable and active, as its loading was in one example reduced to 0.2%. Nevertheless, no attempts were reported on the use of reduced hydrogen pressure and no information was given on the actual speed of the reaction. In addition, Reetz and coworkers reported that the use of BINOL-derived phosphoramidites in combination with enol ester **2.16** afforded only <10% of the product **2.17**.[10] A possible explanation for the slow development in this area might be due to the weaker coordination of the acyl group of the enol ester to the metal as compared to the enamides, where it is well known that this secondary coordination is important for the enantiodiscrimination (Scheme 2.7).[11]



Scheme 2.7 *Substrate chelating features and catalyst-substrate interactions*

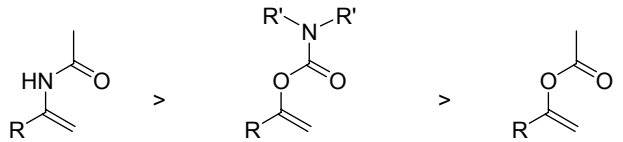
2.3 Earlier work and aim of this study

In view of the excellent results we have previously obtained with BINOL-derived monodentate phosphoramidites (Scheme 2.8) in the Rh-catalyzed hydrogenation of a variety of prochiral olefins **2.1-2.4** (Scheme 2.1),[12,13] we became intrigued with the idea of applying these ligands to the more challenging enol acetates **2.5**.



Scheme 2.8 *General structure of the BINOL-derived monodentate phosphoramidite ligands used in this study*

We also envisaged the use of enol carbamates **2.20**, as it was believed that the increased electron density would improve the binding capabilities of the substrate to the metal center and make it more akin to an enamide (Scheme 2.9).



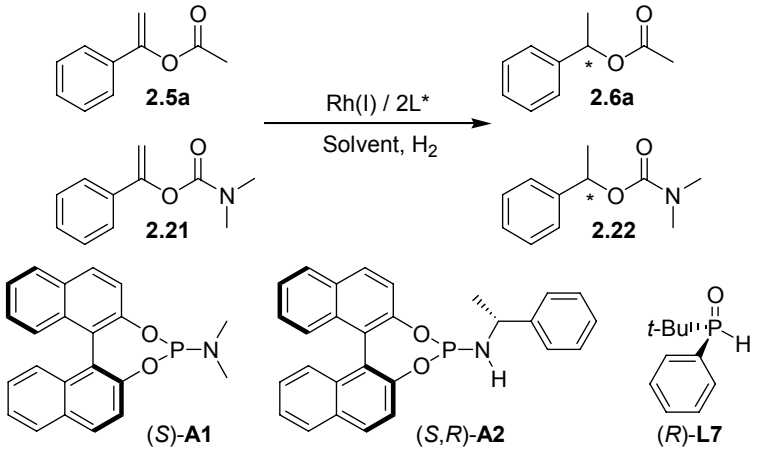
Enamide **2.1** Enol Carbamate **2.20** Enol Acetate **2.5**

Scheme 2.9 *Substrates with bidentate structural features*

Simple 1-phenyl-vinyl acetate (**2.5a**) and 1-phenyl-vinyl *N,N*-dimethylcarbamate (**2.21**) have been tested in a couple of occasions in our research group using Monophos™ (**A1**),[13] (*R*)-α-methylbenzylamine based phosphoramidite (**A2**)[14] and *t*-Bu(Ph)(H)PO (**L7**),[15] as chiral ligands. The not encouraging results are depicted in Table 2.1.

Surprisingly, MonoPhos™ (**A1**, Table 2.1, entry 1) induced a low 8% ee in the hydrogenation of **2.5a** without reaching full conversion. Faster reaction but equally poor enantioselectivity was achieved with ligand **A2** (entry 2). The hydrogenation of enol carbamate **2.21** also afforded **2.22** with rather poor, although definitely higher, enantioselectivities (entries 3 and 4). The use of SPO ligand **L7** seemed to be more promising as 81% ee could be obtained, but the reaction time was very long and improvement on the reactivity could not be obtained. When using this class of chiral ligands, increase in H_2 pressure had a negative effect on both reactivity and enantioselectivity.[16]

Table 2.1 *Previous attempts of asymmetric hydrogenation of enol esters and carbamates using monodentate ligands*



entry	substrate	ligand[a]	Rh(I)	pH_2 (bar)	time (h)	conv.(%)	ee (%)
1	**2.5a**	(*S*)-**A1**[b]	$Rh(COD)_2BF_4$	5	19	95	8
2	**2.5a**	(*S*,*R*)-**A2**[c]	$Rh(COD)_2BF_4$	1	0.25	75	7
3	**2.21**	(*S*)-**A1**[d]	$Rh(COD)_2BF_4$	5	20	>99	17
4	**2.21**	(*S*,*R*)-**A2**[c]	$Rh(NBD)_2BF_4$	5	15	>99	36
5	**2.21**	(*R*)-**L7**[c,e]	$Rh(NBD)_2BF_4$	1	64	>99	81

[a]DCM, rt. [b]S/C= 27. [c]S/C= 100. [d]S/C= 21. [e]EtOAc used as solvent.

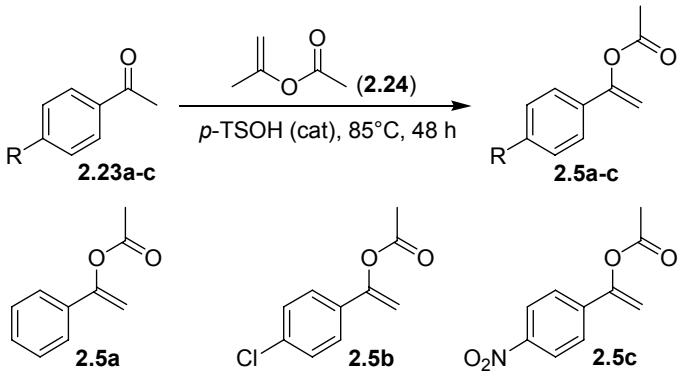
These preliminary results seemed to confirm that enol acetate **2.5a** is a challenging substrate in Rh-catalyzed hydrogenation also using monodentate phosphoramidite

ligands. The outcome of these experiments seemed somehow to contradict with what was reported by Reetz and coworkers,[10] at least in terms of activity, although taking into account that the substrate they refer to is different (**2.16**, Scheme 2.6). Despite the modest results, the Rh-catalyzed asymmetric hydrogenation of enol carbamate **2.21** seemed to hold promise.

The aim of this study was to explore the potential suitability of enol acetates **2.5** and enol carbamates **2.20** as substrates for Rh-catalyzed asymmetric hydrogenation in combination with monodentate phosphoramidites (Scheme 2.8) as chiral ligands.

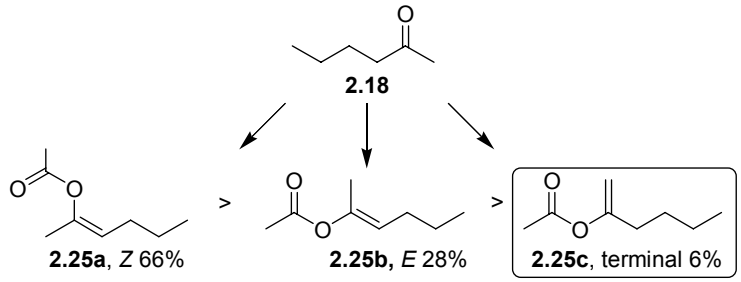
2.4 Synthesis of 1-aryl-vinyl acetates

The most straightforward and generally applied preparation of the aromatic enol acetates **2.5** is the procedure described by House and coworkers.[17] It involves the acid catalyzed enolization of the corresponding ketone **2.23** and capture of the enol with isopropenyl acetate (**2.24**) by transesterification (Scheme 2.10).



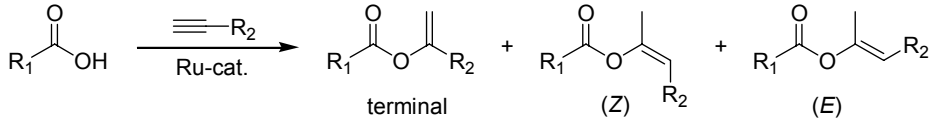
Scheme 2.10 *Synthesis of 1-aryl-vinyl acetates **2.5a-c***

Compounds **2.5a-c** were prepared according to this literature procedure and isolated by fractional distillation under reduced pressure (**2.5a**, **2.5b**) or silica gel flash chromatography (**2.5c**). The reaction relies on the tautomerism between ketones possessing an α-hydrogen and their enols and is particularly suitable for aryl ketones **2.23**, since only one product is obtained from their acylation. Nevertheless, it is of limited scope, as different regioisomers would be obtained whenever dialkyl ketones **2.18** would be used as starting material. An example reported by House is shown in Scheme 2.11.[18]



Scheme 2.11 *Mixture of enol acetates obtained from asymmetric dialkyl ketones*

The terminal enol acetate **2.25c**, the most suitable of these regioisomers for hydrogenation purposes, turned out to be the less abundant product. In this case a valuable alternative would be the regiocontrolled Ru-catalyzed addition of carboxylic acids to alkynes, first reported by Dixneuf and coworkers and more recently by Goossen and coworkers (Scheme 2.12).[19] Ishii and coworkers also reported a similar Ir-catalyzed reaction.[20]

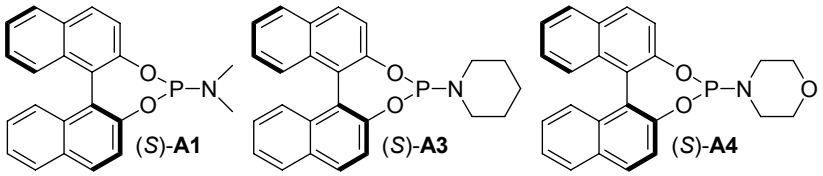


Scheme 2.12 *Regiocontrolled Ru-catalyzed synthesis of enol acetates*

The use of different reaction conditions (ligand and additives) allowed almost exclusively either the less substituted (with terminal double bond) or the more substituted (with internal double bond) products to be obtained. This method is limited only by the availability of the alkynes.

2.5 Asymmetric hydrogenation of 1-aryl-vinyl acetates

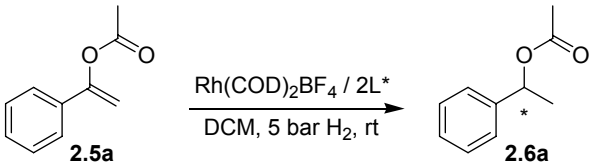
To establish the activity and selectivity of phosphoramidite-based catalysts, initial hydrogenation experiments were performed on the simple 1-phenyl-vinyl acetate (**2.5a**) using three different phosphoramidites **A1**, **A3**, **A4** (Scheme 2.13).



Scheme 2.13 *Monodentate phosphoramidites used for the Rh-catalyzed asymmetric hydrogenation of vinyl acetates **2.5a-c***

MonoPhos™ (**A1**) was used as a reference to the earlier work (Table 2.1). PipPhos (**A3**) and MorfPhos (**A4**) were chosen because they have recently been proven able to improve the already excellent performances of MonoPhos™ for many classes of substrates.[12a] The initial conditions used are those established in the hydrogenation of standard dehydroamino esters (**2.2**) whilst using MonoPhos™ as chiral ligand (Chapter 1) and the results are depicted in Table 2.2.[21]

Table 2.2 *Hydrogenation of 1-phenyl-vinyl acetate (**2.5a**)*



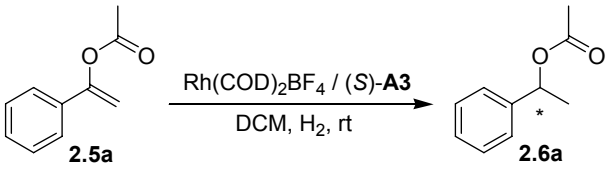
entry	ligand	conv[a,b] (%)	ee[c] (%)
1	(*S*)-**A1**	89	10 (*R*)
2	(*S*)-**A3**	**97**	**90** (*R*)
3	(*S*)-**A4**	90	66 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 1 mol% of catalyst at rt for 16 h. [b]Conversions determined by ^{1}H NMR and GC. [c]ee's determined by chiral GC.

As expected, and in line with the previous results, MonoPhos™ (**A1**) induced only 10% ee without reaching full conversion (Table 2.2, entry 1). On the contrary, an *exciting 90% ee* was obtained by using PipPhos (**A3**, entry 2), whilst ligand **A4** gave again a modest result (entry 3). In all cases, full conversion was not achieved, but luckily the ligand that provided the best enantioselectivity also induced the highest rate. In order to reach full conversion and to determine if higher H_2 pressure would speed up the reaction without compromising the enantioselectivity, experiments at different H_2 pressure were performed (Table 2.3). The same solvent, temperature and catalyst loading were used, and it was decided to concentrate on ligand **A3**, due to its superior performance.

At higher hydrogen pressure, an increase in activity and no decrease in enantioselectivity were observed, in agreement with what was reported for other classes of substrates (**2.1**-**2.4**) using chiral phosphoramidite ligands. According to the H_2 uptake, full conversion was reached in around 8 hours at 10 bar H_2 (Table 2.3, entry 2) and in around 4 hours at 25 bar H_2 (entry 4) using 1 mol% of catalyst.

Table 2.3 *Hydrogenation of 1-phenyl-vinyl acetate (**2.5a**) at different H_2 pressure*

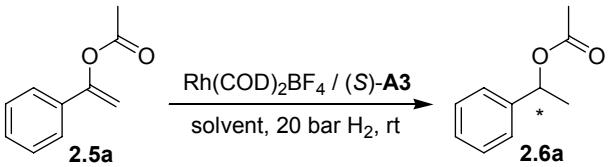


entry	pressure H_2 (bar)	conversion[a,b] (%)	ee[c] (%)
1	5	97	90 (*R*)
2	10	100	90 (*R*)
3	20	100	91 (*R*)
4	25	100	91 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 1 mol% of catalyst at rt for 16 h. [b]Conversions determined by ^{1}H NMR and GC. [c]ee's determined by chiral GC.

A number of experiments using different solvents were performed, in order to determine if this had any influence on reactivity and enantioselectivity. The results are depicted in Table 2.4 and the solvents are listed in order of increasing polarity.

Table 2.4 *Hydrogenation of 1-phenyl-vinyl acetate (**2.5a**) in different solvents*



entry	solvent (polarity index)	conversion[a,b] (%)	ee[c] (%)
1	Toluene (2.4)	7	racemic
2	DCM (3.1)	100	**91** (*R*)
3	*i*-PrOH (3.9)	100	10 (*R*)
4	THF (4.0)	61	racemic
5	EtOAc (4.4)	56	9 (*R*)
6	MeOH (5.1)	92	**26** (*S*)

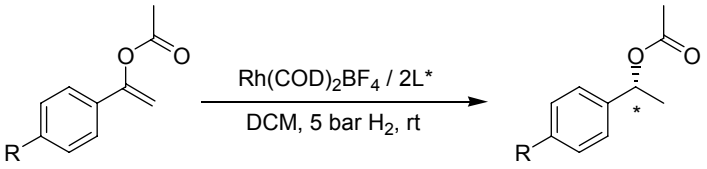
[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 1 mol% of catalyst at rt for 16 h. [b]Conversions determined by ^{1}H NMR and GC. [c]ee's determined by chiral GC. [d]In all cases, the (*S*)-enantiomer of the ligand was used.

The results depicted in Table 2.4 unequivocally established that CH_2Cl_2 was the best in terms of both reactivity and enantioselectivity, similar to most phosphoramidite-based hydrogenations, but the solvent dependency is more

dramatic in the present system (entry 2).[21] The influence of the solvent is very strong as none of the results obtained with the other solvents are even slightly comparable with CH_2Cl_2, as is usually the case in the hydrogenation of other classes of substrates. Moreover, using MeOH as solvent, the opposite enantiomer of **2.6a** (26% ee) was obtained. No relation between the results and the polarity of the solvents could be deduced. A possible explanation for such a clear preference could be found in the lack of competition between CH_2Cl_2 and the substrate in the coordination with the metal complex, compared to all the other solvents. This would confirm the assumption that enol acetates **2.5** have limited coordinating capabilities compared to other classes of substrates, which can be successfully hydrogenated with high enantioselectivities using a variety of catalytic systems.[4]

The other 1-aryl-vinyl acetates synthesized (**2.5b-c**) were also studied using **A3** and **A4** as chiral ligands and CH_2Cl_2 as solvent. The results are listed in Table 2.5.

Table 2.5 *Hydrogenation of 1-aryl-vinyl acetates **2.5b-c***



2.5b, R= Cl
2.5c, R= NO_2

2.6b, R= Cl
2.6c, R= NO_2

entry	substrate	product	ligand	conversion[a,b] (%)	ee[c] (%)
1	**2.5b**	**2.6b**	(*S*)-**A3**	74	78 (*R*)
2	**2.5b**[d]	**2.6b**	(*S*)-**A3**	92	**90** (*R*)
3	**2.5b**	**2.6b**	(*S*)-**A4**	32	29 (*R*)
4	**2.5c**	**2.6c**	(*S*)-**A3**	100	**98** (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 1 mol% of catalyst at rt for 16 h. [b]Conversions determined by ^{1}H NMR and GC. [c]ee's determined by chiral GC. [d]Reaction carried out at 20 bar H_2.

Good selectivity but somewhat lower reactivity was observed using ligand **A3** with 1-*p*-Cl-phenyl-vinyl acetate (**2.5b**). Even using 20 bar H_2 pressure, full conversion was not achieved, but an increase in enantioselectivity was obtained (Table 2.5, entries 1 and 2). Lower reactivity for substrate **2.5b** was previously observed using a Ru-TunaPhos (**L2**) catalyst (48 h).[8c] In contrast, an excellent 98% ee with higher reactivity (entry 4) was obtained with 1-*p*-NO_2-phenyl-vinyl acetate (**2.5c**) even using 5 bar H_2 pressure.

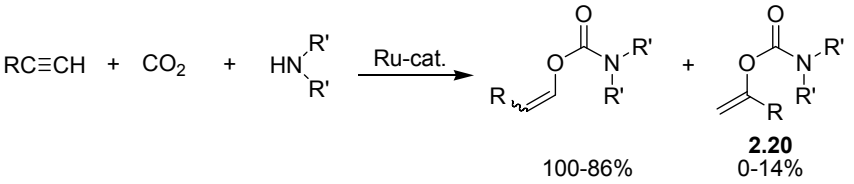
The outcome of this preliminary investigation showed that monodentate phosphoramidites are indeed suitable ligands for the Rh-catalyzed asymmetric hydrogenation of aromatic vinyl carboxylates **2.5a-c**. The use of CH_2Cl_2 as the

solvent and PipPhos (**A3**) as the chiral ligand seemed so far to be essential. Even small structural changes (ligands **A3** and **A4**) induced remarkably different results as shown in Table 2.2 (entries 2 vs 3) and Table 2.5 (entries 1 vs 3).

Subsequently, it was decided to test the assumptions regarding the superiority of vinyl carbamates **2.20** as substrates for asymmetric hydrogenation, due to their supposed higher electron density and therefore better coordination capabilities.

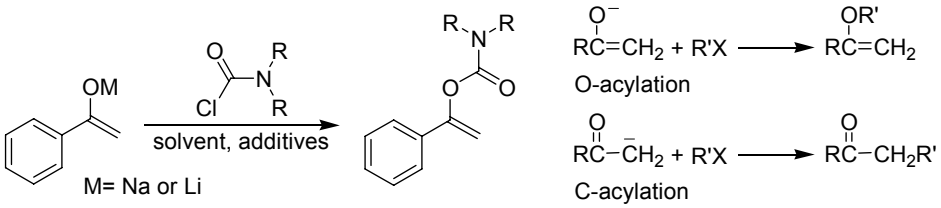
2.6 *Synthesis of 1-phenyl-vinyl N,N*-dialkyl carbamates

Vinyl carbamates are known to be useful intermediates for the preparation of agricultural chemicals, pharmaceutical intermediates or precursors for polymers. Vinyl carbamates have been prepared by addition of amines to vinyl chloroformates, or by dehydrohalogenation of α-halogeno and β-halogenoalkyl carbamates.[22] Nevertheless, all these procedures imply at some stage the use of phosgene. Dixneuf and coworkers reported an interesting alternative synthesis catalyzed by Ru complexes (Scheme 2.14) that unfortunately did not afford the regioisomer **2.20** as the main product, which would be more interesting as substrate for Rh-catalyzed asymmetric hydrogenation.[23]



Scheme 2.14 *Alternative synthesis of vinyl carbamates*

Once more, the most convenient pathway seemed to be the preparation of simple 1-phenyl-vinyl *N,N*-dialkyl carbamates from the corresponding ketones, in this case by reacting their enolates with carbamoyl chlorides (Scheme 2.15).

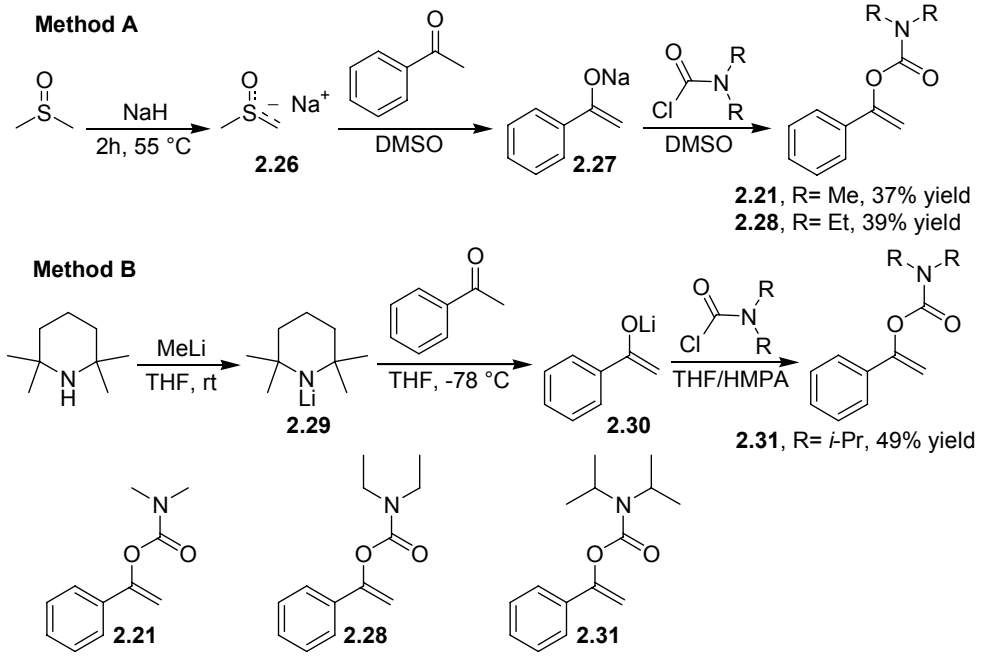


Scheme 2.15 *Vinyl carbamates by quenching of the corresponding enolates*

As the enolate needed to be formed quantitatively by using suitable bases, a number of factors needed to be taken into account. Enolates are ambivalent nucleophiles, so the most obvious side product of this procedure is the dicarbonyl compound derived from competitive C-acylation. Minimization of this side reaction

can be achieved by a proper choice of solvent, co-solvent and metal counter-ion, which parameter also influence the reactivity.[24] Polar aprotic solvents such as DMSO, DMF, *N*-methylpyrrolidone and HMPA are known to be good cation solvators and poor anion solvators. This means that alkali metal counter-ions (Li^+, Na^+, K^+), which are very sensitive to the aggregation state, will be strongly solvated leaving a *pseudo* bare and more reactive enolate. Moreover, O-acylation will be also enhanced by these conditions. On the other side, solvents like THF and DME, in which the enolate is less reactive but more stable, facilitate product work-up, making them a desirable choice. Nevertheless, the reactivity and regioselectivity can be regained by the addition of co-solvents such as HMPA and TMEDA.

Scheme 2.16 describes the two methods adopted for the synthesis of *N,N*-dimethyl (**2.21**), *N,N*-diethyl (**2.28**) and *N,N*-diisopropyl (**2.31**) substituted 1-phenyl-vinyl carbamates, which were obtained in moderate yields.



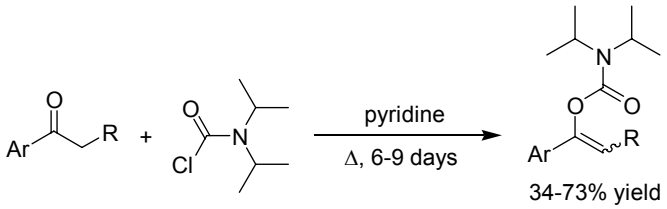
Scheme 2.16 *Synthesis of 1-phenyl-vinyl N,N-dialkyl carbamates*

In the first approach (Scheme 2.16, method A), DMSO was used both as reagent and as solvent. The methyl sulfinyl carbanion **2.26** ("dimsyl anion") was formed by reaction of the solvent with NaH at 55 °C. Corey and coworkers first reported the versatility and reactivity of this base in organic synthesis.[25] It should be noted that the temperature should not exceed 70 °C, otherwise decomposition occurs, which is induced also by prolonged reaction times. Upon addition of acetophenone, the conjugated base of DMSO afforded the corresponding sodium enolate derivative

2.27, which was trapped with the desired carbamoyl chloride affording **2.21** and **2.28**.[15] The moderate yield could be attributed to the competing C-acylation, but also to the formation of a hydroxyl sulfoxide adduct.[25]

In the second approach (Scheme 2.16, method B), a procedure developed by Olofson and coworkers for the synthesis of enol carbonates was adopted and predominant O-acylation was achieved.[26] In this case, the enolate **2.30** was obtained by deprotonation of acetophenone with the H^+ arpoon lithium amide base LiTMP (**2.29**). The crucial point in this case is that the liberated amine should not be more reactive towards the carbamoyl chloride than the enolate. Olofson and coworkers found out that indeed HTMP did not compete in the reaction with the acylation reagent. LiTMP (**2.29**) was conveniently prepared at room temperature by reacting MeLi with HTMP in THF. The enolate **2.30** was formed at -70 °C. After warming the reaction mixture to room temperature and the addition of HMPA, which promoted preferential O-acylation, the enolate **2.30** was reacted with diisopropyl carbamoyl chloride. During the work-up, HTMP and HMPA were efficiently removed by extraction with a pH 4 aqueous citrate solution and the product **2.31** was obtained with an improved 49% yield, compared to **2.21** and **2.28**. As no evident side products were detected, the modest yield was mainly attributed to the lower reactivity of the carbamoyl chloride compared to chloroformates; an observation also reported by Olofson and coworkers.[27]

Hoppe and coworkers described an alternative synthesis of 1-aryl-vinyl *N*,*N*-diisopropyl carbamates (Scheme 2.17).[28] Aryl ketones were reacted with *N*,*N*-diisopropyl carbamoyl chloride in the presence of pyridine at temperatures ≥95 °C for 6 to 9 days (34-73% yields). Although operationally simple, the reaction times are rather long and the scope is not wide.

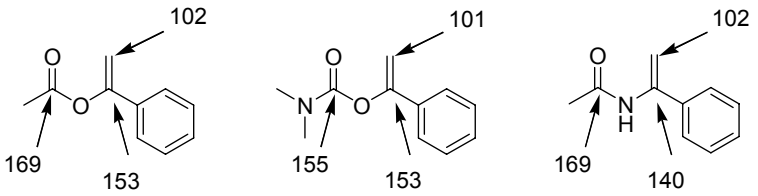


Scheme 2.17 *Alternative synthesis of 1-aryl-vinyl N,N-diisopropyl carbamates*

2.7 Vinyl carbamates as bidentate substrates

As previously mentioned, the importance of the use of chelating substrates in Rh-catalyzed asymmetric hydrogenation is well known. Therefore, the ability of the substrate to coordinate efficiently to the metal center does influence the outcome of the reaction in terms of both reactivity and enantioselectivity. The spectroscopic data depicted in Scheme 2.18 furnish a possible comparison between vinyl acetate **2.5a** and vinyl carbamate **2.21**. The latter was expected to have a more electron-

rich carbonyl moiety. Indeed, the carbonyl signal of **2.21** appeared at higher field in the ^{13}C-NMR compared to **2.5a** indicating that it is more shielded. These values were compared with those of the equivalent *N*-acyl 1-phenyl enamide (**2.32**) reported by Burk and coworkers.[29]



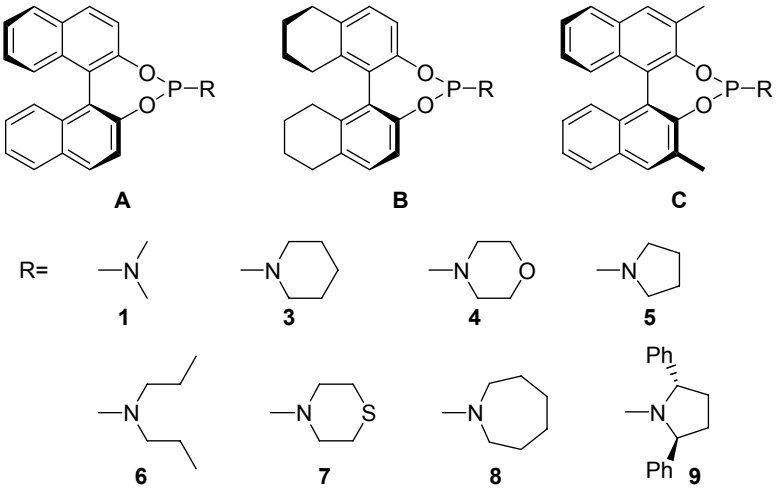
2.5a **2.21** **2.32**

Scheme 2.18 *^{13}C-NMR evidence for electronic differences between 1-phenyl-vinyl acetate (**2.5a**), 1-phenyl-vinyl N,N-dimethyl carbamate (**2.21**) and N-acyl 1-phenyl enamide (**2.32**).*

It was striking to notice that the carbonyl group of the vinyl acetate **2.5a** was the most similar to the carbonyl of the enamide **2.32** (Scheme 2.18, 169.0 vs 168.5 ppm). On the other hand, the value of the ^{13}C-NMR for the α-carbon turned out to be similar between vinyl acetate **2.5a** and carbamate **2.21**, but completely different from the same carbon of the enamide **2.32** (153.2 vs 139.9 ppm). Moreover, there is no significant influence on the β-carbon, which has a similar value in all three compounds (101.4-102.0 ppm). As expected, vinyl acetate **2.5a** turns out to have the most electron-poor carbonyl group and double bond among the molecules considered. On the other side, enamide **2.32** has a more electron-rich α-carbon on the double bond, which should confer to the molecule better binding properties. Conversely, vinyl carbamate **2.21** has a more electron-rich carbonyl moiety, due to the presence of the amino group, which could counterbalance the in principal less efficient binding of the double bond to the metal center, compared to the enamide **2.32**.

2.8 Hydrogenation of 1-phenyl-vinyl *N*,*N*-dialkyl carbamates

At first, 1-phenyl-vinyl *N*,*N*-methyl carbamate (**2.21**) was tested in the Rh-catalyzed asymmetric hydrogenation. In view of the strong influence of the phosphoramidite ligand structure observed in the hydrogenation of aromatic enol acetates **2.5a-c** on both activity and enantioselectivity, more ligands were tested in this part of the investigation (Scheme 2.19).

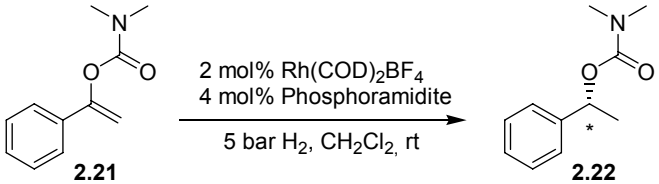


Scheme 2.19 *Monodentate phosphoramidites used in this study*

During this first screening 2 mol% of catalyst loading was used and CH_2Cl_2 was chosen as the solvent. The results are shown in Table 2.6.

Hydrogenation of **2.21** confirmed our assumptions, as evidenced by an increase of enantioselectivity from 90% for **2.6a** to 94% ee for **2.22** using ligand **A3** (Table 2.6, entry 2). Very good activities were obtained in almost all cases, except when a sterically demanding amine (**A9**, entry 8) or BINOL moiety (**C3**, entry 10) were used. The variety of enantioselectivities obtained demonstrates the influence of the ligand structural features. Other heterocyclic ring sizes in the ligand (**A5** and **A8**) resulted in poorer enantioselectivities compared to **A3**. Changing the backbone of the ligand from BINOL (**A3**) to octahydro-BINOL (**B3**) produced no change in the enantioselectivity as 94% ee was achieved in both cases (entries 2 and 9). Under these conditions, in one occasion the reaction was performed at -20 °C (entry 11) affording **2.22** with an increased 97% ee but incomplete conversion. Nevertheless, the activity could be regained by increasing the hydrogen pressure, as this was already demonstrated to have no negative effect on the enantioselectivity (Table 2.3).

Table 2.6 *Asymmetric hydrogenation of 1-phenyl-vinyl N,N-dimethyl carbamate*



2.21 **2.22**

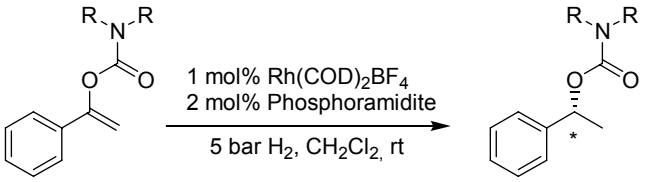
entry	ligand	conversion [a,b] (%)	ee[c,d] (%)
1	**A1**	100	19 (*R*)
2	**A3**	100	**94** (*R*)
3	**A4**	100	**93** (*R*)
4	**A5**	100	14 (*R*)
5	**A6**	100	64 (*R*)
6	**A7**	6	22 (*R*)
7	**A8**	100	76 (*R*)
8	**A9**	3	**92** (*R*)
9	**B3**	100	**94** (*R*)
10	**C3**	77	15 (*R*)
11	**A3**	69[e]	97 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 2 mol% of catalyst at rt for 16 h. [b]Conversions determined by ^{1}H NMR or GC. [c]ee's determined by chiral GC. [d]In all cases, the (*S*)-enantiomer of the ligand was used. [e]Reaction performed at -20 °C.

In view of the superior performance of ligands **A3** and **B3**, they were selected as the ligands of choice for further studies. Table 2.7 shows the results obtained in the hydrogenation of 1-phenyl-vinyl *N,N*-dialkyl carbamates **2.28** and **2.31** varying the substitution pattern on the nitrogen.

A small increase in ee from 94% to 96% was noted in the hydrogenation of the *N,N*-diethyl carbamate **2.28**, with both ligands **A3** and **B3** (entries 3 and 5). An excellent 98% ee (entry 4) was also obtained by decreasing the temperature to -20 °C and, as mentioned before, full conversion was achieved using an higher hydrogen pressure. On the other hand, no further improvement was achieved using substrate **2.31**. It should be emphasized that the catalyst loading for this set of reactions was reduced to 1 mol%, without affecting reactivity or enantioselectivities (entry 1). A closer look at the reaction rate revealed an efficient catalytic system and a substantial difference between ligands **A3** and **B3**, as the reactions were found to be finished in around 4 h and 2 h, respectively.[30]

Table 2.7 *Asymmetric hydrogenation of 1-phenyl-vinyl N,N-dialkyl carbamates*



2.21, R= Me
2.28, R= Et
2.31, R= *i*-Pr

2.22, R= Me
2.33, R= Et
2.34, R= *i*-Pr

entry	substrate	product[a,b]	ligand	ee[c,d] (%)
1	**2.21**[e]	**2.22**	**A3**	94 (*R*)
2	**2.21**	**2.22**	**A3**[h]	95 (*R*)
3	**2.28**[e]	**2.33**	**A3**	**96** (*R*)
4	**2.28**[f]	**2.33**	**A3**	**98** (*R*)
5	**2.28**[g]	**2.33**	**B3**	96 (*R*)
6	**2.31**	**2.34**	**A3**	95 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 1 mol% of catalyst at rt for 16 h. [b]All reactions went to full conversion. [c]ee's were determined by chiral GC. [d]In all cases, the (*S*)-enantiomer of the ligand was used. [e]Reaction complete after 4 h. [f]Reaction carried out at -20 °C and 20 bar H_2. [g]Reaction complete after 2 h. [h]Reaction performed in DCE.

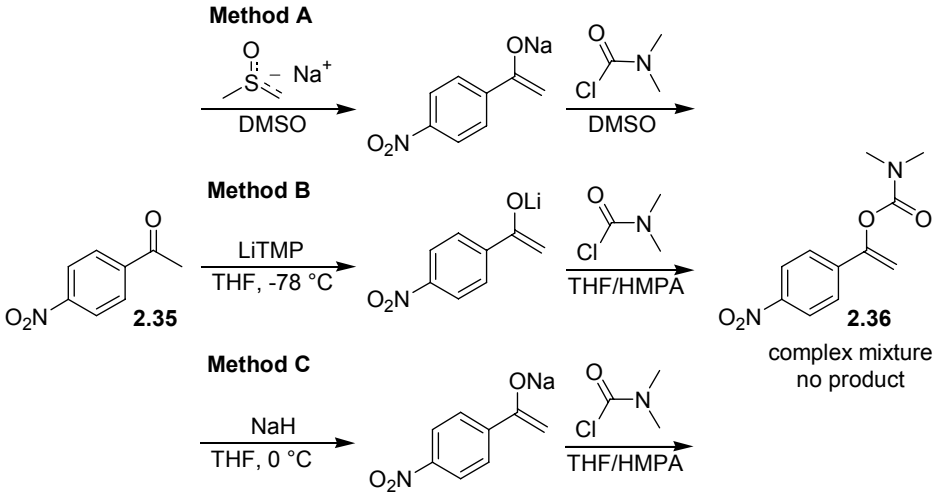
This investigation confirmed our assumption that vinyl carbamates **2.20** are better substrates than vinyl acetates **2.5** (90% ee vs 96% ee). Changes in the substituents on the amine moiety did not seem to have a very significant influence in terms of sterics. It remains to be seen what would have happened with different substitution patterns, as for example more electron rich substituents on the amino group. Nevertheless, it was convenient at this stage to learn that the *N*,*N*-diisopropyl carbamoyl group was not better than the others, as *N*,*N*-diisopropyl carbamoyl chloride is more expensive.

2.9 Expanding the scope of the use of vinyl carbamates

In view of these excellent results, we decided to expand the substrate scope further. Nevertheless, it became immediately clear that also a new synthetic approach was necessary.

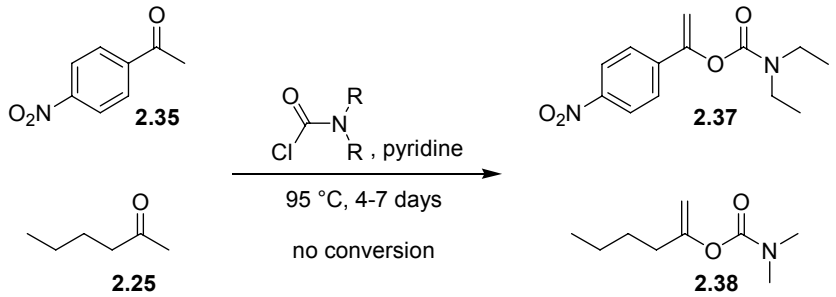
A complex reaction mixture and no product were obtained when using both the previously adopted methods A and B (Scheme 2.20) for the preparation of 1-*p*-NO_2-phenyl-vinyl *N*,*N*-dimethyl carbamate (**2.36**). A modified version of method B

was also attempted. In this case the enolate was directly obtained with NaH but a mixture of THF and HMPA was used as solvent (method C). Also from this approach, only a complex mixture was obtained in which the product was not present. Clearly, the reaction conditions were not selective for the carbonyl group anymore. An involvement of the *para*-nitro functionality of ketone **2.35** seemed to be a reasonable explanation for the complex reaction mixture.



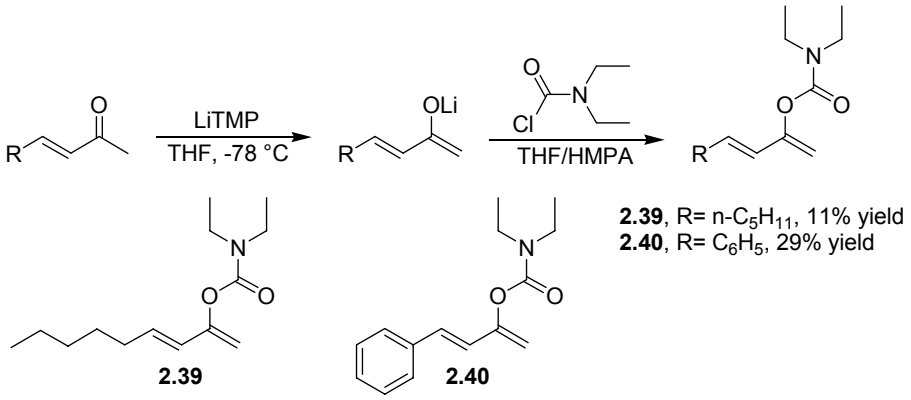
Scheme 2.20 *Attempts for the synthesis of 1-p-NO_2-phenyl-vinyl N,N-dimethyl carbamate (**2.36**)*

The pathway proposed by Hoppe (Scheme 2.17) was also taken into consideration, for the synthesis of 1-*p*-NO_2-phenyl-vinyl *N*,*N*-diethyl carbamate **2.37** (Scheme 2.21). On this occasion, after one week at 95 °C, almost no product was observed. The same methodology was also employed attempting the synthesis of the alkyl vinyl carbamate **2.38**. Again, after 4 days of reaction at 95 °C, no conversion was observed.



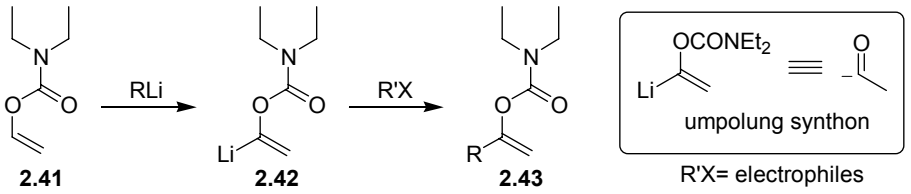
Scheme 2.21 *Attempts for the synthesis of 1-p-NO_2-phenyl-vinyl N,N-diethyl carbamate **2.37** and n-butyl-vinyl N,N-dimethyl carbamate **2.38***

The methodology proposed by Olofson and coworkers[26] was also adopted for the synthesis of dienyl carbamates **2.39** and **2.40** from the corresponding ketones (Scheme 2.22). The products were isolated in modest yields from complex reaction mixtures, showing a possible involvement of the additional double bond. Nevertheless, these compounds were of particular interest as hydrogenation substrates, as they represented the equivalent of the dienyl acetates **2.12a-b** used by Boaz with Rh-DuPHOS (**L3**) as chiral catalyst (Scheme 2.5).[9]



Scheme 2.22 *Synthesis of dienyl carbamates **2.39** and **2.40**.*

The limitations of the syntheses used, stimulated us to look for different approaches that would allow the preparation of a wider range of compounds under milder and more selective conditions. Searching in the literature, we came across a very interesting methodology described by Snieckus and coworkers involving the regiospecific α-lithiation of the simple vinyl carbamate **2.41** (Scheme 2.23).[31]

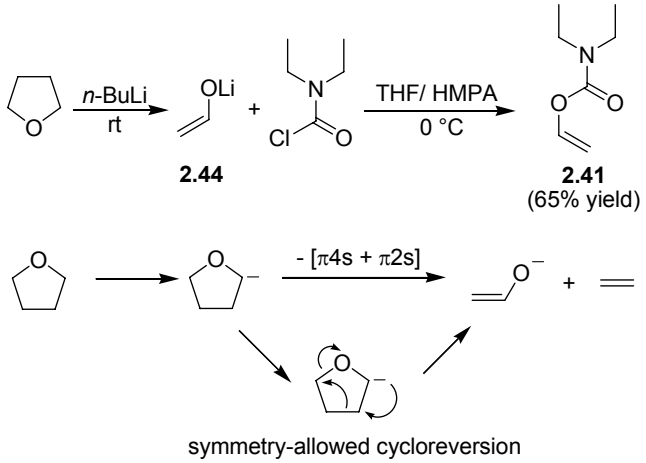


Scheme 2.23 *Enol carbamates **2.43** by regiospecific α-lithiation of enol carbamate **2.41***

The α-metalated vinyl carbamate **2.42** constitutes a readily available and stable acyl anion equivalent in a very simple form, which, in principal, can be trapped with a variety of electrophiles, providing a general synthetic pathway. This approach is based on the principal of umpolung or reversal of polarity, first introduced by Corey and Seebach,[32] which found numerous synthetic applications.[33]

The synthon **2.41** was prepared by trapping the corresponding lithium enolate **2.44**, formed from the fragmentation of THF mediated by *n*-BuLi at room temperature,

with *N*,*N*-diethyl carbamoyl chloride in the presence of HMPA, as described in Scheme 2.24.



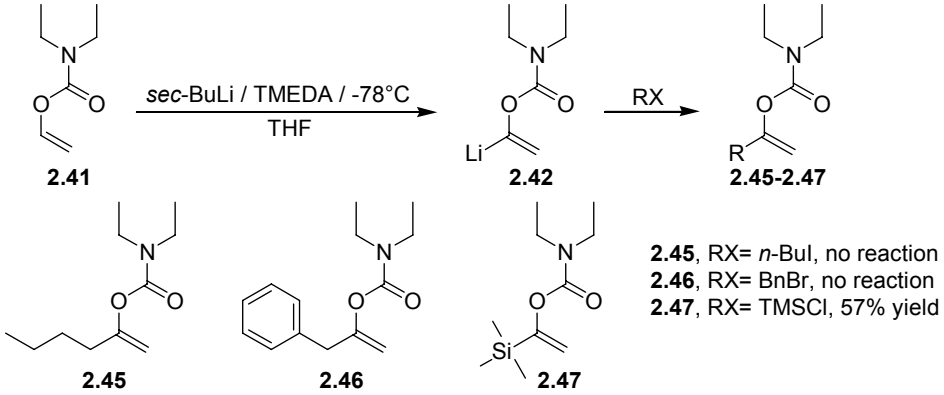
Scheme 2.24 *Synthesis of vinyl N,N-diethylcarbamate **2.41***

The fragmentation, initiated by the deprotonation of THF, is the result of a symmetry-allowed cycloreversion[34] as reported by Bates and coworkers.[35] Cleavage of ethers using organoalkali metal compounds has been reported in the literature.[36] Moreover, this side reaction has been the object of NMR studies in view of possible synthetic applications of the metal enolates formed.[35,37] In a report by Jung and Blum, the lithium enolate of acetaldehyde **2.44** was obtained on a preparative scale and used in O-acylation and O-silylation reactions.[38] Modifications of these literature procedures allowed the preparation on a multigram scale of **2.41** isolated in 65% yield by silica gel flash chromatography. The initial use of 3 equivalents of *N*,*N*-diethyl carbamoyl chloride afforded a conversion of 78%. However, the excess of reagent still present complicated the purification of the product, resulting in a much lower isolated yield of the pure product (25-40%). Eventually, the use of 1.1 equivalents of carbamoyl chloride seemed to be a good compromise between reactivity and purification issues. Alternatively, the product could be isolated by distillation under reduced pressure but again the product was contaminated by the presence of residual carbamoyl chloride, so column chromatography was preferred.[39]

According to the literature procedure, vinyl carbamate **2.41** was lithiated in the α-position at -78 °C using *sec*-BuLi in the presence of TMEDA in THF, following a reverse addition protocol (Scheme 2.23).[31] The reaction of electrophiles with the resulting species **2.42** would then lead to the desired α-substituted vinyl carbamates **2.43** with alkyl halides serving as effective reaction partners.

This seemed appealing, as we were interested in the possibility of preparing in a regioselective manner terminal alkyl vinyl carbamates to test in the asymmetric

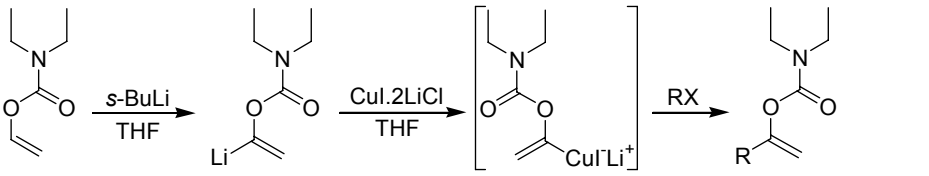
hydrogenation reaction. Nevertheless, in our hands following the reported protocol, reaction with *n*-butyl iodide or benzyl bromide did not afford the expected products **2.45** and **2.46** and the starting material **2.41** was instead recovered (Scheme 2.25). Using the more reactive TMSCl, the expected product **2.47** was obtained in 57% isolated yield.[40] This at least guaranteed that the α-lithio vinyl carbamate **2.42** was indeed formed.



Scheme 2.25 *Synthesis of N,N-diethyl vinyl carbamates **2.45-2.47***

The procedure implied that the electrophile was added at -78 °C and maintained at this temperature for one hour. Subsequently the reaction mixture was allowed to warm to room temperature, where it was quenched after 15 minutes with ammonium chloride. A number of attempts was made in order to increase the reactivity by changing some of the reaction conditions. For example, the temperature at which *n*-butyl iodide was added was increased to -60 °C, or the reaction mixture was maintained at -78 °C for a couple of hours. Alternatively, after reaching room temperature, the reaction mixture was stirred for longer periods or more *n*-butyl iodide was used. Unfortunately, none of these attempts led to any improvement. Moreover, there was the risk that higher temperatures might cause instability of the lithium intermediate **2.42**.

Transmetallation to α-cuprate vinyl carbamates (**2.48**) seemed at this point to be a possible solution as it would allow temperatures more suitable for the alkylation reducing the risk of degradation. The procedure adopted is described in Scheme 2.26. Percy and coworkers used this approach in order to increase the reactivity of the α-lithio fluoro-vinyl carbamate equivalent of **2.42**, which resulted to be much less reactive than **2.42**.[41]



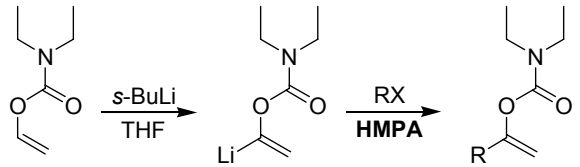
2.41 **2.42** **2.48**

2.45, RX= *n*-BuI, traces
2.46, RX= BnBr, 40% yield

Scheme 2.26 *Synthesis by transmetallation to vinyl copper carbamate **2.48***

The copper salt CuI·2LiCl used for the transmetallation was prepared according to a procedure described by Reetz and coworkers and has the advantage of being soluble in THF.[42] After addition of the copper salt, as a 1M THF solution at -78 °C, to the α-lithio vinyl carbamate **2.42**, the reaction mixture was stirred for 1 hour before raising the temperature to 0 °C. Upon addition of the electrophile, stirring was continued for two hours at 0 °C and overnight at room temperature. In this way, vinyl carbamate **2.46** was isolated in 40% yield, but only traces of product **2.45** were detected in the crude mixture by ^{1}H-NMR and TLC. The α-cupro vinyl carbamate **2.48** did not show better reactivity toward simple alkyl electrophiles. It was decided to abandon this synthetic pathway without trying any optimization of the reaction conditions.

As mentioned before (see page 50) HMPA is known to enhance the reactivity of enolates in THF by weakening the coordination between the metal and the enolate itself. The aim was to achieve better reactivity and regioselectivity during O-acylation. It was decided to use HMPA as an additive also on this occasion in order to increase the reactivity of **2.42** by solvation of the metal (Scheme 2.27).[43]



2.41 **2.42**

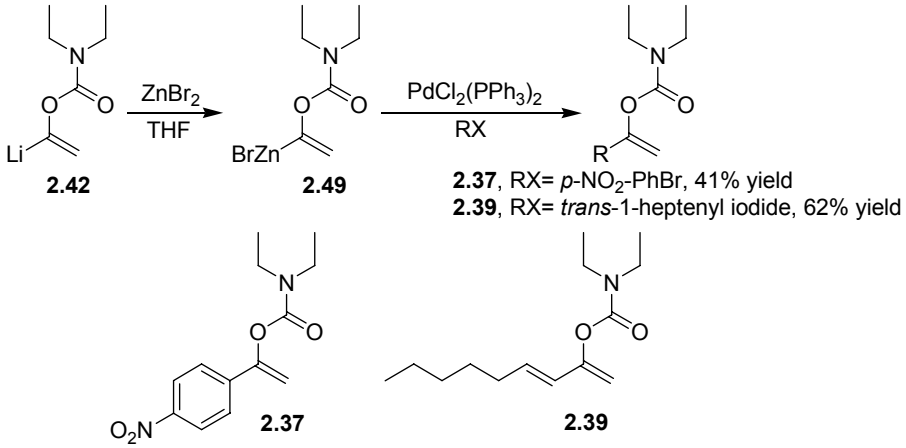
2.45, RX= *n*-BuI, 75% yield
2.46, RX= BnBr, 52% yield

Scheme 2.27 *Synthesis of vinyl carbamates **2.45** and **2.46** in the presence of HMPA*

The α-lithio vinyl carbamate **2.42** was prepared as described before and n-butyl iodide was added at -78 °C followed by HMPA. Immediately a change of color was noticed. The temperature was increased to room temperature and the reaction was quenched after 2 hours. This modified protocol proved to be very efficient as vinyl carbamate **2.45** was *finally* isolated in 75% yield. The reaction was also performed using benzyl bromide yielding **2.46** with an improved 52% yield.

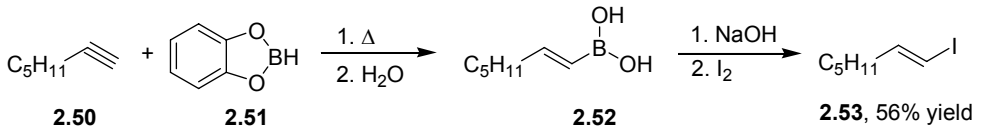
Snieckus and coworkers reported that the same synthon **2.41** could also be used for the preparation of aryl or heteroaryl derivatives.[44] The α-lithio carbamate **2.42**

was in this case transmetallated with $ZnBr_2$. The α-Zn species **2.49** obtained was used in Negishi cross coupling reactions (Scheme 2.28). Instead of the reported $PdCl_2(dppf)$, the already available $PdCl_2(PPh_3)_2$ was used as catalyst during the reaction. This allowed the preparation of the *p*-NO_2-phenyl-vinyl carbamate **2.37**, which could not be prepared before. Moreover, using these reaction conditions dienyl carbamate **2.39** was obtained with an improved 62% yield.



Scheme 2.28 *Synthesis of p-NO_2-phenyl (**2.37**) and alkenyl (**2.39**) N,N-diethyl vinyl carbamates*

The *trans*-1-heptenyl iodide (**2.53**) used as starting material for the preparation of **2.39** was not commercially available. However, it was conveniently synthesized by stereospecific and regioselective conversion of 1-heptyn (**2.50**) *via* hydroboration as described in Scheme 2.29.[45]



Scheme 2.29 *Synthesis of trans-1-heptenyl iodide (**2.53**) via hydroboration*

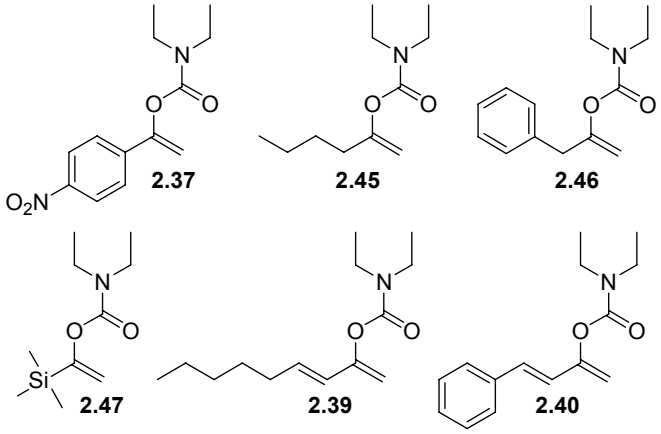
The catechol ester of *trans*-1-heptenylboronic acid **2.52** was exclusively obtained from the reaction between catechol borane (**2.51**) and 1-heptyne (**2.50**) at 70 °C. The ester was subsequently hydrolyzed to the *trans*-1-heptenylboronic acid (**2.52**). After isolation, boronic acid **2.52** was reacted first with an aqueous solution of NaOH, after which an ethereal solution of I_2 was added dropwise. The desired product **2.53** was obtained in 56% yield after purification by distillation. The sequential steps in this second part of the synthesis were particularly important, as too fast addition of I_2 yielded in a first attempt a mixture of *cis* and *trans* products.[46]

In spite of the problems initially encountered, α-lithio vinyl carbamate **2.42** proved to be a general and versatile synthon for a mild and regioselective preparation of differently α-substituted vinyl *N*,*N*-diethyl carbamates.

The reactions involving the use of vinyl carbamate **2.41** (Scheme 2.27 and Scheme 2.28) are mild, selective and the crude mixtures resulting are rather uncomplicated.

2.10 Hydrogenation of a range of α-substituted vinyl *N*,*N*-diethyl carbamates

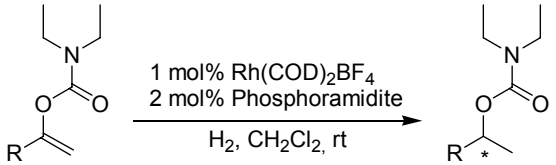
The α-substituted vinyl *N*,*N*-diethyl carbamates prepared (Scheme 2.30) were subsequently tested as substrates in the Rh-catalyzed asymmetric hydrogenation. Once more, the best ligands (**A3**, **B3**) and solvent (CH_2Cl_2) were used and the results are depicted in Table 2.8.



Scheme 2.30 *α-Substituted vinyl N,N-diethyl carbamates used in Rh-catalyzed asymmetric hydrogenation*

An excellent 98% ee (entry 1) was obtained for substrate **2.37**, confirming also for enol carbamates the importance of an electron-withdrawing group on the aromatic moiety. Moreover, this substituent had a beneficial influence also on the activity, as the reaction was finished after around 1 h. According to Burk, the presence of electron-withdrawing groups enhances metal olefin binding, resulting in higher rates and enantioselectivities.[8j]

Table 2.8 *Hydrogenation of α-substituted vinyl N,N-diethyl carbamates*



entry	substrate	product [a,b]	ligand	H_2 (bar)	ee[c,d] (%)
1	**2.37**	**2.54**	**A3**	5	**98** (*R*)
2	**2.45**	**2.55**	**A3**	5[e]	64 (*S*)
3	**2.45**	**2.55**	**A3**	25	63 (*S*)
4	**2.45**	**2.55**	**B3**	10	69 (*S*)
5	**2.46**	**2.56**[f]	**A3**	5	73 (nd)
6	**2.46**	**2.56**	**A3**	10	73 (nd)
7	**2.47**	**2.57**[f]	**A3**	5	43 (nd)
8	**2.39**	**2.58**	**A3**	10	**97** (*R*)
9	**2.39**	**2.58**	**A3**	15	97 (*R*)
10	**2.40**	**2.59**	**A3**	15	76 (*R*)
11	**2.40**	**2.59**	**B3**	10	77 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate and 1 mol% of catalyst at rt for 16 h. [b]Conversions determined by ^{1}H-NMR or GC, all reactions went to completion. [c]ee's were determined by chiral GC. [d]In all cases, the (*S*)-enantiomer of the ligand was used. [e]Conversion 41%. [f]The absolute configuration has not been established.

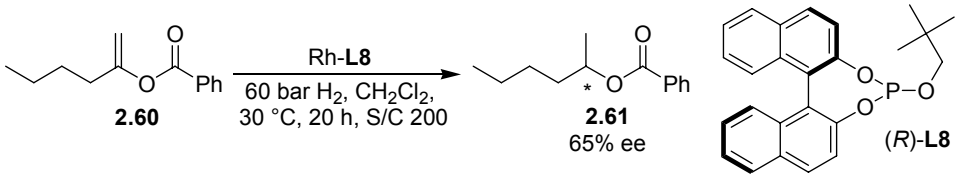
As shown for substrate **2.46** (entry 5), the presence of a benzyl group causes a decrease in enantioselectivity (73% ee) although full conversion was achieved with 5 bar H_2 (in approximately 10 h) using the catalyst based on ligand **A3**. Using higher hydrogen pressure afforded the product **2.56** with identical enantioselectivity but in approximately 6 h (entry 6). When the substituent is an alkyl group (substrate **2.45**), a further decrease in enantioselectivity (63% ee, entry 3) was observed and a higher pressure was necessary in order to reach full conversion. It was pleasing to see an increase both in terms of enantioselectivity (69% ee) and reactivity (only 10 bar H_2 were used) for this substrate when ligand **B3** was employed (entry 4). The results using substrates **2.45** and **2.46** are comparable with those obtained by using DuPHOS (**L3**) on similar enol acetates (**2.10a-b**, Scheme 2.5).[9] Interestingly the sterically hindered substrate **2.47** could also be hydrogenated to full conversion (entry 7) although the enantioselectivity was modest. To our surprise, an excellent 97% ee was achieved for the 2-dienyl substrate **2.39** (entry 8), and only 10 bar H_2

was necessary to achieve complete conversion to the product **2.58**. A similar phenomenon was previously observed by using DuPHOS (**L3**) on a similar enol acetate (**2.12a**, Scheme 2.5).[9] On the other hand, lower enantioselectivity was observed for the 2-dienyl carbamate **2.40** (entries 10 and 11). It should be mentioned that in both cases the catalytic system showed very good selectivity as the hydrogenation proceeded leaving the extra internal double bond intact. In one case (entry 9), increasing further the hydrogen pressure seemed to make the reaction slightly less regioselective, as a small amount (< 5%) of over hydrogenated product was also observed.

2.11 Conclusions and outlook

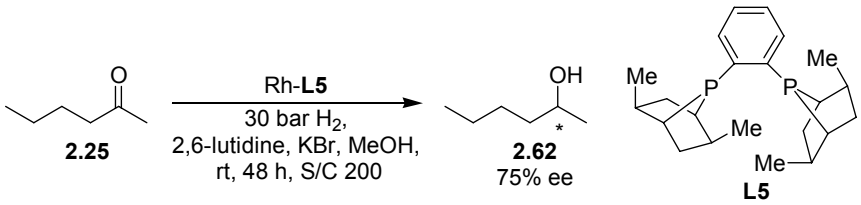
In conclusion, we have shown that monodentate phosphoramidites, in particular PipPhos (**A3**) and its octahydro analogue (**B3**), are excellent ligands for the rhodium-catalyzed asymmetric hydrogenation of aromatic enol acetates, aromatic enol carbamates and 2-dienyl carbamates with excellent enantioselectivities up to 98%. Fast reactions were achieved (TOF up to 100 h^{-1}, 5 bar H_2), making the combination of enol carbamates and monodentate phosphoramidites very competitive compared to the existing systems.

High enantioselectivities with aliphatic vinyl carbamates, such as **2.45**, **2.46**, remain an interesting challenge. The results even if not extremely exciting (69% and 73% ee) are comparable to what was obtained using Duphos (64% and 77% ee) and the catalyst appeared to be rather active as full conversion was achieved using 5 and 10 bar H_2 pressure, respectively. Also Reetz and coworkers reported moderate results (up to 65% ee) in the hydrogenation of vinyl carboxylate **2.60** using simple monodentate phosphite ligands such as **L8** without any match/mismatch combination provided by the carbohydrate backbone (Scheme 2.31), demonstrating that the structure of the ligand is extremely important.



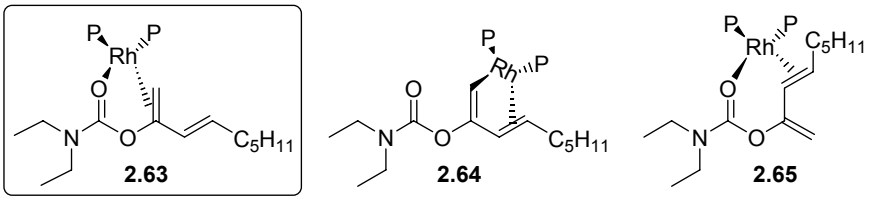
Scheme 2.31 *Hydrogenation of vinyl carboxylate **2.60** with chiral phosphite **L8***

Before the carbohydrate based ligands, described by Reetz (Scheme 2.6), were employed the best result reported was obtained by direct hydrogenation of 2-hexanone (**2.25**) using Rh-PennPhos catalyst and affording **2.62** with 75% ee (Scheme 2.32).[47] Nevertheless, the system was rather slow, as 48 hours and 30 bar hydrogen pressure were necessary to reach 96% conversion.



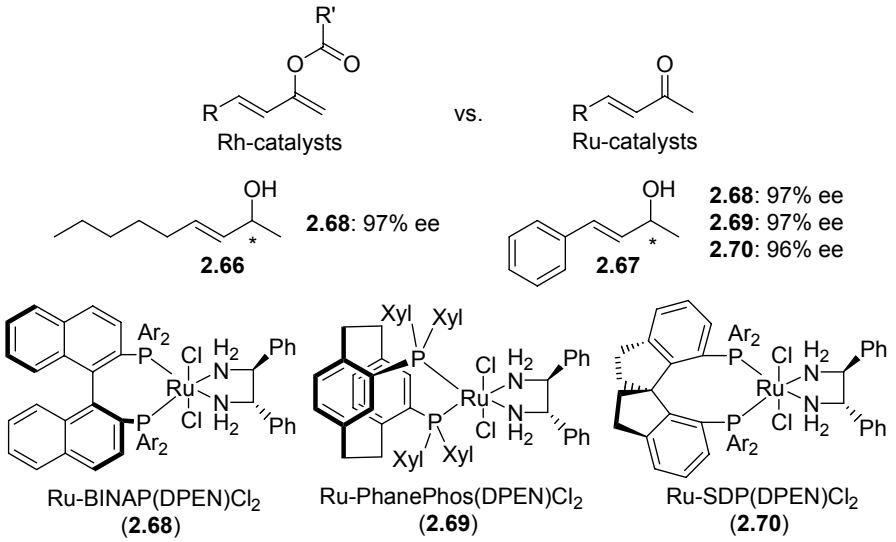
Scheme 2.32 *Asymmetric hydrogenation of 2-hexanone (**2.25**)*

Very interestingly, the hydrogenation of 2-dienyl carbamate **2.39** afforded the product **2.58** with an excellent 97% ee. The regioselectivity would suggest, as depicted in Scheme 2.33, a distinct preferential coordination of the terminal double bond and the carbamoyl group to the metal center (**2.63**) compared to the other possible complexes **2.64** and **2.65**.



Scheme 2.33 *Possible coordination between Rh and dienyl carbamate **2.39***

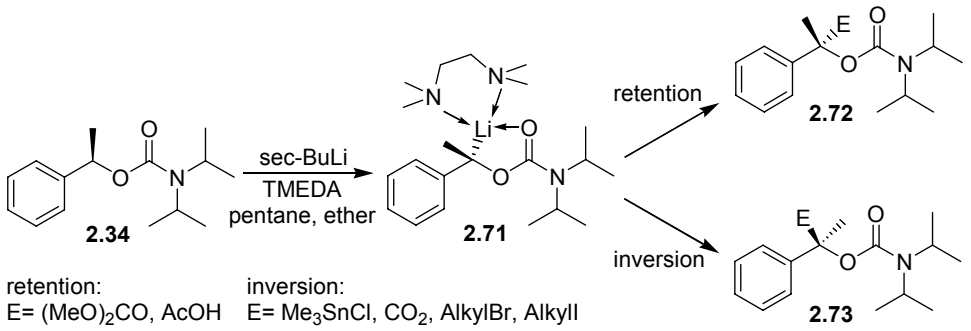
Furthermore, the high enantioselectivity would also suggest that the poorer results obtained with **2.45** might be mainly the result of steric rather than electronic effects, especially considering that also for **2.59** a lower enantiomeric excess was achieved (77% ee). This would suggest that the flexibility of the alkyl chain could be detrimental for the enantioselectivity. In this respect, it would be interesting to see which results the use of the *cis*-dienyl equivalent of **2.39** would provide. Very good enantioselectivities with these dienol substrates were reported not only by Boaz (Scheme 2.5) and by us, but were found also in the hydrogenation of the corresponding α,β-unsaturated ketones using ruthenium catalysts (Scheme 2.34).[48]



Scheme 2.34 *Hydrogenation of α,β-unsaturated ketones to allyl alcohols*

It seems likely that the substrate scope could be expanded substantially, by screening a wider variation of ligands and even combination of different ligands.[5,49] Phosphoramidites being monodentate ligands and having a modular structure are perfect candidates for a combinatorial approach. The dramatic influence of the chiral ligand was already underlined by the results provided by MonoPhos™ (**A1**) and PipPhos (**A3**) in the hydrogenation of both **2.5a** and **2.21**. In this respect, the chair conformation of the piperidine ring might provide a more defined or constrained chiral environment that suits this class of substrates more than others previously tested. For example, it has already been observed by ^{31}P-NMR studies that by mixing $Rh(COD)_2BF_4$ with 2 equivalents of MonoPhos™ (**A1**) more than one complex can be observed containing different rhodium to ligand ratios.[13]

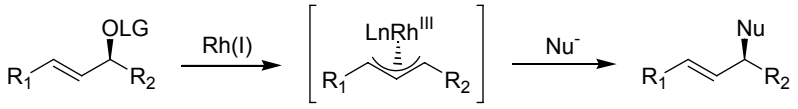
The assumption that vinyl carbamates should be better substrates than vinyl acetates was confirmed by the results obtained for **2.5a** (90% ee) and **2.31** (96% ee). Moreover, the use of a carbamate as stereo-directing group allowed the regioselective preparation of a diverse number of substrates starting from the very useful and simple compound **2.41**. The versatility of the carbamate moiety could be further exploited due to the configurational stability in apolar solvents of the related lithium derivatives, an example of which is reported in Scheme 2.35.[50]



Scheme 2.35 *Tertiary chiral esters from chiral hydrogenation product **2.34***

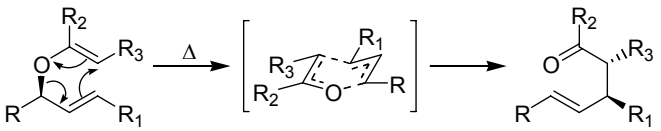
As demonstrated by Hoppe and coworkers,[51] tertiary lithium intermediate **2.71** derived from benzyl carbamate **2.34** undergoes electrophile-dependent stereo-divergent substitutions, affording tertiary chiral esters **2.72** and **2.73**. Furthermore, the substitution with Me_3SnCl proceeds with inversion to provide access to the other enantiomer of the products.[52]

Moreover, optically active allylic carbamates **2.58** and **2.59** could be used for further transformations involving the remaining unsaturation. In a generic sense, the chiral allylic alcohol or its carbamate derivative might serve as stereo-directing and possible metal binding site for all kind of reactions involving a double bond (Heck reaction, hydroformylation, epoxidation, etc.).[53] An intriguing idea would be to employ the enantiomerically enriched allylic carbamates in regioselective and enantiospecific Rh-catalyzed allylic substitutions reactions using the carbamate as leaving group.[54,55]



Scheme 2.36 *Regioselective, enantiospecific Rh-catalyzed allylic substitutions reactions*

Finally, chiral allylic alcohol derivatives can undergo selective Claisen rearrangement *via* a chair transition state affording products with an extra stereogenic center, which still retain a double bond (Scheme 2.37).[56]



Scheme 2.37 *Claisen rearrangement of chiral allylic alcohol derivatives*

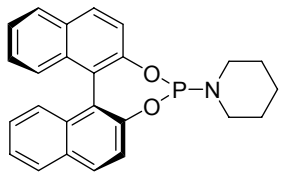
2.12 Experimental section

General remarks

All reactions were performed in a dry nitrogen atmosphere using standard techniques. Solvents were reagent grade, dried and distilled before use following standard procedures.[57]

^{1}H-NMR and ^{13}C-NMR spectra were recorded at room temperature in $CDCl_3$ on a Varian VXR300 (300 MHz) spectrometer. Chemical shifts were determined relative to the residual solvent peaks ($CDCl_3$, δ = 7.26 ppm for proton, δ = 77 ppm for carbon). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz) and integration. Mass spectra were recorded on an AEI-MS-902 mass spectrometer. Melting points were measured on a Büchi B-545 melting point apparatus and are uncorrected. Optical rotations were measured on a Schmidt-Haensch Polartronic MH8 polarimeter. Enantiomeric excesses and conversions were determined by capillary GC analysis on a HP 6890 or 5890 gas chromatograph equipped with a flame ionization detector. All the monodentate phosphoramidite ligands have been previously described and are generally available in our laboratories.[58]

1-(3,5-Dioxa-4-phospha-cyclohepta[2,1-*a*;3,4-*a*']dinaphthalen-4-yl)-piperidine (*S*)-A3:[59]

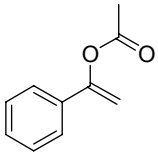


(*S*)-Bis-β-naphthol (5 g, 17.5 mmol) in PCl_3 (20 mL) was heated under reflux for 8 h. Excess of PCl_3 was removed by distillation under reduced pressure (20 mbar). The residual solid was subjected to azeotropic distillation with toluene (2 x 10 mL) and dried in vacuum. The resulting yellowish foam was dissolved in toluene (25 mL) and added dropwise at 0 °C to a solution of triethylamine (5.3 mL, 38.5 mmol, 2.2 eq) and distilled piperidine (1.9 mL, 19.3 mmol, 1.1 eq) in dry THF (37.5 mL). The reaction mixture was then allowed to warm to room temperature and it was stirred overnight. The reaction mixture was diluted with dry diethyl ether (125 mL), filtered over a plug of silica and washed with diethyl ether (125 mL). The solvent was removed under reduced pressure and the crude mixture was purified by silica gel flash chromatography (heptane / ethyl acetate 8:1) affording the desired product as a white solid (89%).
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.87-8.03 (m, 4H), 7.52-7.15 (m, 8H), 3.04-2.81 (m, 4H), 1.58-1.26 (m, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 150.0 (s), 149.6 (s), 132.8 (s), 132.6 (s), 131.3 (s), 130.7 (s), 130.2 (d), 129.7 (d), 128.3 (d), 128.2 (d), 127.0 (d), 126.9 (d), 126.0 (d, 2C), 124.7 (d), 124.4 (d), 123.9 (s), 122.2 (d), 122.08 (s), 122.05 (d), 45.5 (t), 45.1 (t), 27.0 (t), 26.9 (t), 24.9 (t). ^{31}P (162 MHz, $CDCl_3$) δ 145.3.

Synthesis of 1-aryl-vinyl acetates (2.5a-c):[17]

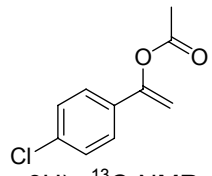
A mixture of ketone (38.6 mmol) and *p*-toluenesulfonic acid monohydrate (0.81 g, 4.3 mmol) was stirred for 48 h at 85 °C in isopropenyl acetate (25 mL). The solvent was then removed under reduced pressure, the crude mixture was dissolved in EtOAc (100 mL), washed with a saturated aqueous solution of $NaHCO_3$, H_2O, brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure.

1-Phenylvinyl acetate (2.5a):[60]



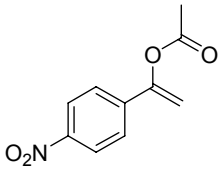
Conversion 69%. Purification by vacuum distillation (120 °C, 20 mmHg) afforded the desired product as a colorless liquid (64%). Alternatively, purification by silica gel flash chromatography (pentane / ether, 9:1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.52-7.45 (m, 2H), 7.41-7.31 (m, 3H), 5.50 (d, *J*= 2.2 Hz, 1H), 5.05 (d, *J*= 2.2 Hz, 1H), 2.29 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 169.0 (s), 152.7 (s), 133.6 (s), 130.4 (d), 128.9 (d, 2C), 124.7 (d, 2C), 102.0 (t), 20.9 (q).

1-*p*-Cl-Phenylvinyl acetate (2.5b):



Conversion 70%. Purification by vacuum distillation (150 °C, 20 mmHg) afforded the desired product as a colorless liquid (63%). Alternatively, purification by silica gel flash chromatography (pentane / ether 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 (dt, *J*= 8.8, 2.2 Hz, 2H), 7.32 (dt, *J*= 8.8, 2.2 Hz, 2H), 5.46 (d, *J*= 2.2 Hz, 1H), 5.05 (d, *J*= 2.2 Hz, 1H), 2.27 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 168.9 (s), 151.9 (s), 134.8 (s), 132.8 (s), 128.7 (d, 2C), 126.2 (d, 2C), 102.7 (t), 20.9 (q).

1-*p*-NO_2-Phenylvinyl acetate (2.5c):[61]



Conversion 62%. Purification by flash column chromatography (heptane / ethyl acetate, 14:1) afforded the desired product as a solid (60%). M.p. = 51.2-52.5 °C (Lit. 51-52 °C) ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.22 (dt, *J*= 8.8, 2.2 Hz, 2H), 7.62 (dt, *J*= 8.8, 2.2 Hz, 2H), 5.65 (d, *J*= 2.6 Hz, 1H), 5.26 (d, *J*= 2.6 Hz, 1H), 2.31 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 168.7 (s), 162.8 (s), 151.0 (s), 140.5 (s), 125.7 (d, 2C), 123.9 (d, 2C), 106.1 (t), 20.9 (q).

Synthesis of 1-phenyl-vinyl N,N-dialkyl carbamates:

Method A[62]

Sodium hydride (8.80 g, 0.220 mol, 60% suspension in oil) was added in portions to DMSO (500 ml, freshly distilled under reduced pressure from calcium hydride). After stirring for 2 h at 50 °C hydrogen evolution ceased and the mixture was cooled to room temperature. To the gray solution the ketone (0.200 mol) in 50 mL DMSO was added dropwise in 30 min, the addition being slightly exothermic and changing the color of the solution to yellow. This solution was left stirring for 15 min before the *N*,*N*-dialkyl carbamoyl chloride (20.3 mL, 0.220 mol) in 50 ml DMSO

was added dropwise in 30 min, while maintaining room temperature. After stirring for an additional 15 min, water (500 mL) was carefully added to the orange solution. The mixture was extracted with heptane (5 x 500 mL) and the combined extracts were washed with brine and dried over magnesium sulfate, followed by removal of the solvent under reduced pressure.

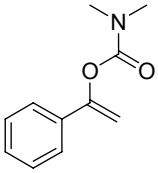
Method B[63]

Lithium 2,2,6,6-tetramethyl-piperidine was prepared by adding dropwise MeLi (0.022 mol, ca. 1.6 M in ether) to a solution of 2,2,6,6-tetramethyl-piperidine (3.10 g, 0.022 mol) in THF (20 mL) in order to accommodate the methane evolution. After an additional 10 min, the solution was cooled to -78 °C and the ketone (0.021 mol) in THF (10 mL) was added dropwise over 20 min. Stirring at -78 °C was continued for another 15 min, after which time the enolate solution was warmed to room temperature and diluted with 40 mL of HMPA. At this point the *N,N*-dialkyl carbamoyl chloride (3.60 g, 0.022 mol) was added to the reaction mixture. Stirring was continued for two hours. The mixture was poured into 50 mL of aqueous 10% citric acid (buffered to pH 4 with 50% aq. NaOH) and pentane was added (50 mL). After separation, the aqueous phase was extracted with pentane (2 x 50 mL) and the combined organic layers washed with an aqueous $NaHCO_3$ solution, water, brine and dried with sodium sulfate, followed by removal of the solvent under reduced pressure.

Method C

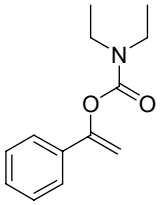
A solution of the ketone (0.021 mol) in 10 mL of THF (with an additional catalytic amount of MeOH) was added dropwise to a solution of NaH (1.06 g, 0.022 mol, 50% suspension in oil) in 20 ml of THF at 0 °C. After 40 min, the yellow reaction mixture was allowed to reach room temperature and it was stirred for 3 hours, until no further evolution of gas was observed. At this point, a solution of the *N,N*-dialkyl carbamoyl chloride (0.032 mol, 1.5 eq.) in 4 mL HMPA was added and the mixture was stirred overnight. After the addition of water (50 mL), the organic layer was separated. The aqueous layer was extracted with ether (3 x 25 mL) and the combined organic layers were washed with water, brine and dried over sodium sulfate. The solvent was then removed under reduced pressure.

1-Phenylvinyl *N,N*-methyl carbamate (2.21):



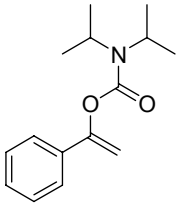
Prepared according to method A. Purification by column chromatography (heptane / ethyl acetate 4:1) afforded the product as a colorless oil (37%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.97 (s, 3H), 3.11 (s, 3H), 5.03 (d, *J*= 1.8 Hz, 1H), 5.42 (d, *J*= 1.8 Hz, 1H), 7.29-7.50 (m, 5H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.1 (s), 153.2 (s), 134.9 (s), 128.5 (d), 128.3 (d, 2C), 124.7 (d, 2C), 101.4 (t), 36.6 (q), 36.3 (q). MS, *m/z (%)*: 191 (M^+, 34%); HRMS for $C_{11}H_{13}NO_2$, calcd: 191.095, found: 191.095.

1-Phenylvinyl *N,N*-diethyl carbamate (2.28):



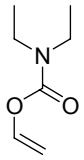
Prepared according to method A. Purification by flash chromatography (pentane / ether 9:1) afforded the product as a colorless oil (39%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.48 (d, *J*= 6.6 Hz, 2H), 7.40-7.28 (m, 3H), 5.41 (d, *J*= 1.5 Hz, 1H), 5.03 (d, *J*= 1.5 Hz, 1H), 3.45 (q, *J*= 6.6 Hz, 2H), 3.35 (q, *J*= 6.6 Hz, 2H), 1.27 (t, *J*= 6.6 Hz, 3H), 1.17 (t, *J*= 6.6 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 154.5 (s), 153.4 (s), 135.3 (s), 128.6 (d), 128.4 (d, 2C), 124.9 (d, 2C), 101.4 (t), 42.0 (t), 41.9 (t), 14.3 (q), 13.3 (q). MS, *m/z (%)*: 219 (M^+, 24.4%); HRMS for $C_{13}H_{17}NO_2$, calcd: 219.126, found: 219.125.

1-Phenylvinyl *N,N*-diisopropyl carbamate (2.31):[64]



Prepared according to method B. Purification by flash chromatography (pentane / ether 9:1) afforded the product as a pale yellow solid (49%). M.p. = 60-62 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.20-7.60 (m, 5H), 5.40 (d, *J*= 1.8 Hz, 1H), 4.98 (d, *J*= 1.8 Hz, 1H), 4.01 (br sept, *J*= 6.6, 6.9 Hz, 2H), 1.33 (br, 6H), 1.27 (br, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 154.6 (s), 153.5 (s), 135.4 (s), 128.5 (d), 128.4 (d), 124.9 (d), 101.3 (t), 46.2 (d, 2C), 21.5 (q, 2C), 20.5 (q, 2C). MS, *m/z (%)*: 247 (M^+, 17.4%); HRMS for $C_{15}H_{21}NO_2$, calcd: 247.157, found: 247.158.

Vinyl *N,N*-diethyl carbamate (2.41):[65,66]



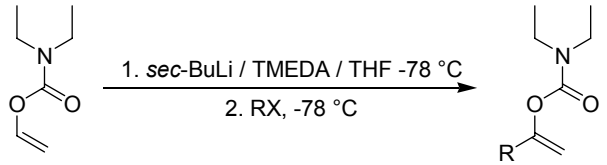
A solution of *n*-BuLi (0.032 mol, ca. 1.6 M in hexane) in THF (20 mL) was allowed to stir at room temperature overnight. The solution was then cooled to 0 °C and *N*,*N*-diethyl carbamoyl chloride (4.75 g, 0.035 mol) in HMPA (20 mL) was added. The solution was allowed to stir at room temperature overnight, during which the color changed to red. The reaction was quenched with a saturated aqueous solution of ammonium chloride and after extraction with ether (50 ml) the organic layer was washed with water, brine and dried with sodium sulfate. After removal of the solvent under reduced pressure, purification by flash chromatography (pentane / ether 9:1) afforded the product as a colorless liquid (65%). Alternatively, the product was purified by distillation at 25 °C and 1 mmHg (Lit. bp 41 °C at 3 mmHg). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.21 (dd, J_{cis}= 6.3 Hz, J_{trans}= 13.8 Hz, 1H), 4.73 (d, J_{trans}= 13.8 Hz, J_{gem}= 1.5 Hz, 1H), 4.39 (d, J_{gem}= 1.5 Hz, J_{cis}= 6.3 Hz, 1H), 3.29 (q, *J*= 7.2 Hz, 4H), 1.13 (t, *J*= 7.2 Hz, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 150.3 (s), 140.1 (d), 92.3 (t), 39.5 (t), 38.9 (t), 11.6 (q), 10.7 (q). MS, *m/z (%)*: 143 (M^+, 18.9%); HRMS for $C_7H_{13}NO_2$, calcd: 143.095, found: 143.094.

Synthesis of α-substituted vinyl N,N-diethyl carbamates via transmetallation with CuI·2LiCl:

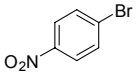
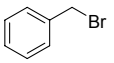
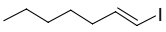
A solution of vinyl *N*,*N*-diethyl carbamate **2.41** (0.40 g, 2.79 mmol) in THF (8 mL) was added dropwise to a THF solution (18 mL) of *sec*-BuLi (2.92 mmol, ca. 1.3 M in hexane) and TMEDA (0.45 mL, 2.98 mmol) at -78 °C. The reaction mixture was

allowed to stir at -78 °C for 1 h. In the mean time LiCl (336 mg, 8 mmol, flamed dried under vacuum and stored over P_2O_5) and CuI (760 mg, 4 mmol) were stirred in THF (4 mL, 1M) until a complete clear solution was obtained (10-15 min). Part of this solution (2.95 mL, 2.95 mmol) was added to the reaction mixture that turned from colorless to bright yellow. The solution was allowed to reach 0 °C over a period of 15 min., after which time RX was added. After stirring at 0 °C for additional two hours, the temperature was increased to room temperature and stirring was continued overnight. The solution was subsequently quenched with a saturated aqueous solution of ammonium chloride (20 mL), extracted with ether (3 x 50 mL) and the combined organic layers were washed with water, brine, dried over sodium sulfate and the solvent was removed under reduced pressure.

Table 2.9 ***General procedures for synthesis of substrates α-substituted vinyl N,N-diethyl carbamates*:**[67,68]



2.41

RX	Procedure	Product
	Procedure E	**2.37**
	Procedure D	**2.45**
	Procedure D	**2.46**
	Procedure D No HMPA used	**2.47**
	Procedure E	**2.39**

General procedure D

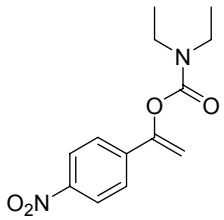
A solution of vinyl *N,N*-diethyl carbamate **2.41** (0.40 g, 2.79 mmol) in THF (8 mL) was added dropwise to a THF solution (18 mL) of *sec*-BuLi (2.92 mmol, ca. 1.3 M in hexane) and TMEDA (0.45 mL, 2.98 mmol) at -78 °C. The reaction mixture was allowed to stir at -78 °C for 1 h, after which a solution of RX (3.17 mmol) in HMPA

(5 mL) was added. After stirring for an additional 15 min. at -78 °C, the solution was allowed to reach room temperature and it was stirred for an additional 2 h. The solution was subsequently quenched with a saturated aqueous solution of ammonium chloride (20 mL), extracted with ether (3 x 50 mL) and the combined organic layers were washed with water, brine and dried over sodium sulfate. After removal of the solvent under reduced pressure, the crude products were purified by flash chromatography (mixtures of pentane / ether) affording the desired compounds.

General procedure E

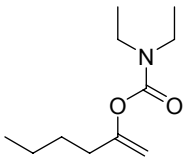
To a stirred solution of vinyl *N*,*N*-diethyl carbamate **2.41** (0.60 g, 4.2 mmol) in THF (20 mL) at -78 °C *sec*-BuLi (4.6 mmol, ca. 1.3 M in hexane) was added. After 1h, a solution of $ZnBr_2$ (1.13 g, 5 mmol, 1.2 eq) in THF (10 mL) was added dropwise. The pale yellow reaction mixture was stirred for an additional 15 min and then allowed to reach room temperature. At this point a solution of $[(C_6H_5)_3P]_2PdCl_2$ (140 mg, 0.21 mmol, 5 mol%) and RX (6.4 mmol, 1.5 eq) in THF (20 mL) was added; the reaction mixture turned from pale yellow to dark orange and it was allowed to stir at room temperature overnight. The reaction was then quenched with a saturated aqueous solution of ammonium chloride (20 mL), extracted with ether (3 x 50 mL) and the combined organic layers were washed with water, brine and dried over sodium sulfate. Purification by flash chromatography (mixtures of pentane / ether) afforded the desired products.

1-(4-Nitro-phenyl)vinyl *N,N*-diethylcarbamate (2.37):



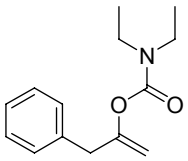
yellow oil (41%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J*= 8.7 Hz, 2H), 7.60 (d, *J*= 8.7 Hz, 2H), 5.56 (d, *J*= 2.1 Hz, 1H), 5.23 (d, *J*= 2.1 Hz, 1H), 3.46 (q, *J*= 6.9 Hz, 2H), 3.33 (q, *J*= 6.9 Hz, 2H), 1.27 (t, *J*= 6.9 Hz, 3H), 1.16 (t, *J*= 6.9 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 153.4 (s), 151.6 (s), 147.7 (s), 141.6 (s), 125.7 (d, 2C), 123.8 (d, 2C), 105.3 (t), 42.3 (t), 41.9 (t), 14.3 (q), 13.3 (q). MS, *m/z (%)*: 264 (M^+, 4.9%); HRMS for $C_{13}H_{16}N_2O_4$, calcd: 264.111, found: 264.110.

1-Butylvinyl *N,N*-diethylcarbamate (2.45):



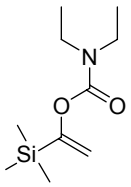
colorless liquid (75%).^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.69 (s, 1H), 4.64 (s, 1H), 3.30 (br, 4H), 2.24 (t, *J*= 6.9 Hz, 2H), 1.52-1.27 (m, 4H), 1.15 (t, *J*= 7.2 Hz, 6H), 0.90 (t, *J*= 7.2 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 156.9 (s), 153.9 (s), 99.9 (t), 41.7 (t, 2C), 33.3 (t), 28.7 (t), 22.1 (t), 14.2 (q), 13.8 (q), 13.4 (q). MS, *m/z (%)*: 199 (M^+, 7.7%); HRMS for $C_{11}H_{21}NO_2$, calcd: 199.157, found: 199.157.

1-Benzylvinyl *N,N*-diethylcarbamate (2.46):



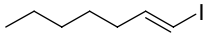
colorless liquid (52%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.10-7.32 (m, 5H), 4.77 (s, 1H), 4.60 (s, 1H), 3.56 (s, 2H), 3.24 (br q, *J*= 6.9 Hz, 2H), 3.07 (br q, *J*= 6.9 Hz, 2H), 1.09 (t, *J*= 6.9 Hz, 3H), 0.90 (t, *J*= 6.9 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.8 (s), 152.0 (s), 137.3 (s), 129.2 (d, 2C), 128.3 (d, 2C), 126.5 (d), 101.7 (t), 41.9 (t), 41.6 (t), 40.1(t), 13.8 (q), 13.3 (q). MS, *m/z (%)*: 233 (M^+, 3.9%); HRMS for $C_{14}H_{19}NO_2$, calcd: 233.142, found: 233.143.

1-Trimethylsilanylvinyl *N,N*-diethylcarbamate (2.47):[69]



colorless liquid (57%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.37 (s, 1H), 5.00 (s, 1H), 3.28 (q, *J*= 7.2 Hz, 4H), 1.13 (t, *J*= 7.2 Hz, 6H), 0.15 (s, 9H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.0 (s), 152.2 (s), 113.1 (t), 41.8 (t, 2C), 14.1 (q), 13.5 (q), -0.1 (q, 3C). MS, *m/z (%)*: 215 (M^+, 5.4%); HRMS for $C_{10}H_{21}NO_2Si$, calcd: 215.134, found: 215.135.

Trans-1-heptenyl iodide (2.53):[70]

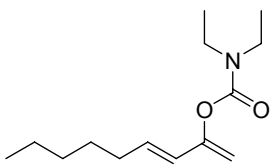


the catechol ester of 1-heptenylboronic acid was formed by stirring 1-heptyne (4.81 g, 0.05 mol) and catecholborane (6.00 g, 0.05 mol) at 70 °C for 2 h. After this time the mixture was cooled to room temperature and stirred with 50 mL of water for 2 h in order to achieve the hydrolysis of the ester. The resulting mixture was cooled to 0 °C and the white solid, *trans*-1-hexylboronic acid, was collected by filtration and washed free of the catechol using ice-cold water. The boronic acid was then dissolved in ether (50 mL) and cooled to 0 °C. An aqueous solution of sodium hydroxide (50 mL, 3 N) was then added, followed by the dropwise addition of a solution of I_2 (15.2 g, 0.06 mol) in ether (150 mL). The mixture was allowed to stir at 0 °C for about 30 min. The excess iodine was destroyed with aqueous thiosulfate solution, the ether solution was separated, washed with water and brine, then dried over sodium sulfate. After removing the solvent, Kugelrohr distillation (55 °C at 2 mmHg) afforded the product as a slightly pink liquid (56%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.51 (dt, *J*= 14.1, 7.2 Hz, 1H), 5.97 (d, *J*= 14.1 Hz, 1H), 2.04 (q, *J*= 7.2 Hz, 2H), 1.45-1.20 (m, 6H), 0.88 (t, *J*= 6.6 Hz, 3H).

^{13}C-NMR (50 MHz, $CDCl_3$) δ 146.7 (d), 74.2 (d), 36.0 (t), 31.1 (t), 28.0 (t), 22.4 (t), 13.9 (q).

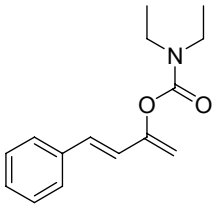
(*E*)-1-Methylene-oct-2-enyl *N,N*-diethylcarbamate (2.39):



colorless liquid (62%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.98 (d, *J*= 15.6 Hz, 1H), 5.79 (dt, *J*= 15.3, 7.2 Hz, 1H), 4.83 (s, 1H), 4.78 (s, 1H), 3.45-3.28 (br, 4H), 2.10 (q, *J*= 7.2 Hz, 2H), 1.47-1.09 (m, 12H), 0.88 (t, *J*= 6.9 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 153.6 (s), 152.3 (s), 132.2 (d), 124.9 (d), 102.8 (t), 42.0 (t), 41.6 (t), 32.1, 31.4 (t), 28.6

(t), 22.5 (t), 14.2 (q), 14.0 (q), 13.3 (q). MS, *m/z (%)*: 239 (M^+, 11.0%); HRMS for $C_{14}H_{25}NO_2$, calcd: 239.189, found: 239.190.

(*E*)-1-Methylene-3-phenyl-allyl *N,N*-diethylcarbamate (2.40):



yellowish oil (29%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38-7.17 (m, 5H), 6.67 (d, J_{trans}= 16.2 Hz, 1H), 6.60 (d, J_{trans}= 16.2 Hz, 1H), 5.03 (d, *J*= 1.5 Hz, 1H), 4.95 (d, *J*= 1.2 Hz, 1H), 3.56-3.25 (m, 4H), 1.23 (t, *J*= 7.2 Hz, 3H), 1.15 (t, *J*= 6.9 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 153.6 (s), 152.3 (s), 136.2 (s), 129.4 (d), 128.6 (d, 2C), 128.0 (d), 126.8 (d, 2C), 123.7 (d), 105.6 (t), 42.1 (t), 41.8 (t), 14.3 (q), 13.4 (q). MS, *m/z (%)*: 245 (M^+, 15.9%); HRMS for $C_{15}H_{19}NO_2$, calcd: 245.142, found: 245.141.

Hydrogenation Procedure.

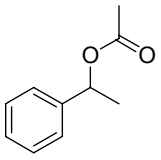
Hydrogenations were performed in an Endeavor™ multireactor autoclave,[71] where the eight reactors are equipped with glass reaction vessels and stirring paddles. In a typical Endeavor run each vessel was charged open to air with $Rh(COD)_2BF_4$ (2 µmol, 1 mol%), monodentate phosphoramidite (4 µmol, 2 mol%) and substrate (200 µmol). Solvent was added (4 ml), the glass liners were placed in the reactors and the system was closed. After repetitive purging with N_2 (3 × 2.5 bar) the system was pressurized with hydrogen and the reaction mixtures were stirred at room temperature overnight with 750 rpm. The conversion of the reactions was monitored following H_2 consumption. The reactions were stopped *via* release of H_2 pressure. The resulting mixture was filtered over a short silica column and subjected to conversion (^{1}H-NMR) and enantiomeric excess determination (capillary GC).

Enantiomeric Excess Determinations.

To ensure accurate ee determination, racemic products of **2.56** and **2.57** were prepared by hydrogenation of **2.46** and **2.47** using 10% Pd/C (10%) in MeOH under 1 bar of H_2 for 16 h. Racemic products of **2.6a-c**, **2.22**, **2.33**, **2.34**, **2.54** and **2.55** were prepared by hydrogenation of **2.5a-c**, **2.21**, **2.28**, **2.31**, **2.37** and **2.45** using $Rh(COD)_2BF_4$ and 3 equivalents of PPh_3 ligand in CH_2Cl_2 under H_2 pressure. Racemic products of **2.58** and **2.59** were prepared by reduction of the corresponding ketones with $NaBH_4$ and carbamoylation of the resulting racemic alcohols. Absolute configurations were determined by comparison with: the GC literature values of identical compounds (**2.6a-c**),[72] the GC retention time of commercially available enantiopure alcohol **2.74** after carbamate removal (**2.22**, **2.33**, **2.34**), the GC retention time of commercially available enantiopure alcohol after carbamoylation (**2.55**), the HPLC literature value of the corresponding saturated alcohol **2.75** after carbamate removal (**2.59**),[73] or assigned by analogy through chiral GC elution order (**2.54**, **2.58**). For products **2.56** and **2.57** the absolute configuration was not determined.

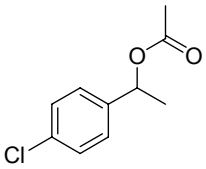
Hydrogenation products data.

1-Phenylethyl acetate (2.6a):[74]



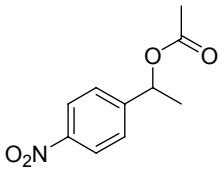
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.37-7.26 (m, 5H), 5.89 (q, *J*= 6.6 Hz, 1H), 2.08 (s, 3H), 1.54 (d, *J*= 6.6 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 170.3 (s), 141.6 (s), 128.5 (d, 2C), 127.8 (d), 126.1 (d, 2C), 72.3 (d), 22.2 (q), 21.3 (q).
Enantiomeric excess determination: β-DEX 120 column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 125 °C, $T_{det/inlet}$ = 150 °C, t_S = 10.39 min, t_R = 10.92 min, t_{SM} = 17.74 min.

1-(4-Chloro-phenyl)-ethyl acetate (2.6b):[75]



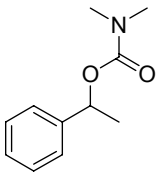
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34-7.26 (m, 4H), 5.84 (q, *J*= 6.4 Hz, 1H), 2.07 (s, 3H), 1.51 (d, *J*= 6.4 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 170.2 (s), 140.2 (s), 133.6 (s), 128.7 (d, 2C), 127.5 (d, 2C), 71.6 (d), 22.1 (q), 21.3 (q).
Enantiomeric excess determination: β-DEX 120 column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 145 °C, $T_{det/inlet}$ = 180 °C , t_S = 13.65 min, t_R = 14.25 min, t_{SM} = 20.24 min.

1-(4-Nitro-phenyl)-ethyl acetate (2.6c):[76]



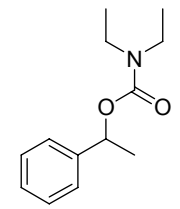
^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J*= 8.8 Hz, 2H), 7.50 (d, *J*= 8.8 Hz, 2H), 5.92 (q, *J*= 6.6 Hz, 1H), 2.11 (s, 3H), 1.55 (d, *J*= 6.6 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 170.0 (s), 149.0 (s), 134.2 (s), 126.7 (d, 2C), 123.8 (d, 2C), 71.2 (d), 22.2 (q), 21.1 (q).
Enantiomeric excess determination: β-DEX 120 column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 170 °C, $T_{det/inlet}$ = 250 °C , t_S = 20.78 min, t_R = 21.25 min, t_{SM} = 26.27 min.

1-Phenylethyl *N,N*-dimethyl carbamate (2.22):



^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.40-7.17 (m, 5H), 5.76 (q, *J*= 6.6 Hz, 1H), 2.90 (s, 3H), 2.86 (s, 3H), 1.49 (d, *J*= 6.6 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.3 (s), 142.6 (s), 128.4 (d, 2C), 127.5 (d), 125.8 (d, 2C), 73.1(d), 35.9 (q, 2C), 22.9 (q). MS, *m/z (%)*: 193 (M^+, 17%); HRMS for $C_{11}H_{15}NO_2$, calcd: 193.110, found: 193.110. $[\alpha]^{20}_D$ +5.0 (*c* 1.09, $CHCl_3$, 94% ee).
Enantiomeric excess determination: CP Chirasil-L-Val column (25 m × 0.25 mm × 0.25 µm). Init. Temp.: 100 °C, 10 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 75:1, t_R = 9.76 min, t_S = 10.12 min, t_{SM} = 13.17 min.

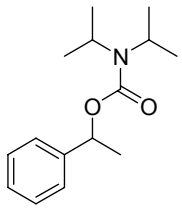
1-Phenylethyl *N,N*-diethyl carbamate (2.33):



^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.37-7.13 (m, 5H), 5.82 (q, *J*= 6.6 Hz, 1H), 3.29 (q, *J*= 6.9 Hz, 4H), 1.53 (d, *J*= 6.6 Hz, 3H), 1.12 (t, *J*= 6.9 Hz, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.2 (s), 142.7 (s), 128.4 (d, 2C), 127.4 (d), 125.8 (d, 2C), 72.7 (d), 41.4 (t, 2C), 22.9 (q), 13.9 (q, 2C). MS, *m/z (%)*: 221 (M^+, 16.2%); HRMS for $C_{13}H_{19}NO_2$, calcd: 221.142, found: 221.142. $[\alpha]^{20}_D$ +4.6 (*c* 1.39, $CHCl_3$, 96% ee).

Enantiomeric excess determination: CP Chirasil-L-Val column (25 m × 0.25 mm × 0.25 µm). Init. Temp.: 100 °C, 20 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 75:1, t_R = 20.42 min, t_S = 20.64 min, t_{SM} = 24.66 min.

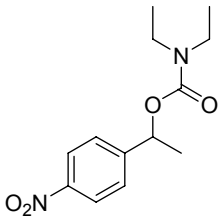
1-Phenylethyl *N,N*-diisopropyl carbamate (2.34):[77]



^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34-7.16 (m, 5H), 5.80 (q, *J*= 6.6 Hz, 1H), 4.35-3.55 (br, 2H), 1.50 (d, *J*= 6.6 Hz, 3H), 1.32-1.03 (br, 12H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.0 (s), 142.8 (s), 128.3 (d, 2C), 127.4 (d), 126.0 (d, 2C), 72.6 (d), 45.7 (d, 2C), 22.8 (q), 20.8 (q, 4C). MS, *m/z (%)*: 249 (M^+, 10.0%); HRMS for $C_{15}H_{23}NO_2$, calcd: 249.173, found: 249.174.

Enantiomeric excess determination: CP Chirasil-L-Val column (25 m × 0.25 mm × 0.25 µm). Init. Temp.: 100 °C, 20 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 75:1, t_R = 23.44 min, t_S = 23.58 min, t_{SM} = 26.62 min.

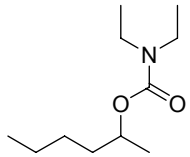
1-(4-Nitro-phenyl)-ethyl *N,N*-diethyl carbamate (2.54):



^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.18 (d, *J*= 8.7 Hz, 2H), 7.48 (d, *J*= 8.7 Hz, 2H), 5.85 (q, *J*= 6.9 Hz, 1H), 3.30 (br, 4H), 1.53 (d, *J*= 6.9 Hz, 3H), 1.12 (br, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 154.7 (s) 152.1 (s), 150.2 (s), 126.5 (d, 2C), 123.8 (d, 2C), 71.7 (d), 41.8 (t), 41.3 (t), 22.7 (q), 14.1 (q), 13.4 (q). MS, *m/z (%)*: 266 (M^+, 21.5%); HRMS for $C_{13}H_{18}N_2O_4$, calcd: 266.127, found: 266.127. $[\alpha]^{20}_D$ -22.7 (*c* 0.90, $CHCl_3$, 98% ee).

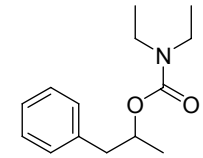
Enantiomeric excess determination: CP Chirasil-L-Val column (25 m × 0.25 mm × 0.25 µm). Init. Temp.: 140 °C, 20 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 25:1, t_R = 25.36 min, t_S = 25.48 min, t_{SM} = 28.07 min.

2-Hexyl *N,N*-diethyl carbamate (2.55):



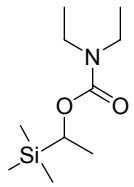
^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.85-4.72 (m, 1H), 3.24 (br, *J*= 5.4 Hz, 4H), 1.55-1.22 (m, 6H), 1.18 (d, *J*= 6.0 Hz, 3H), 1.06 (t, *J*= 6.9 Hz, 6H), 0.87 (br, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.8 (s), 71.3 (d), 41.2 (t, 2C), 36.0 (t), 27.6 (t), 22.5 (t), 20.3 (q), 14.0 (q), 13.8 (q, 2C). MS, *m/z (%)*: 201 (M^+, 17.0%); HRMS for $C_{11}H_{23}NO_2$, calcd: 201.173, found: 201.172. $[\alpha]^{20}_D$ +13.1 (*c* 0.75, $CHCl_3$, 63% ee). Enantiomeric excess determination: CP Chirasil Dex CB column (25 m × 0.25 mm × 0.25 µm). Init. Temp.: 100 °C, 20 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 70:1, t_R = 20.13 min, t_S = 20.39 min, t_{SM} = 22.89 min.

2-Phenylpropyl *N,N*-diethyl carbamate (2.56):



^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.28-7.08 (m, 5H), 5.04-4.92 (m, 1H), 3.18 (br, 4H), 2.90 (dd, *J*= 13.5, 6.6 Hz, 1H), 2.73 (dd, *J*= 13.8, 6.6 Hz, 1H), 1.17 (dd, *J*= 6.3, 1.5 Hz, 3H), 1.02 (br, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.4 (s), 137.9 (s), 129.5 (d, 2C), 128.2 (d, 2C), 126.2 (d), 71.8 (d), 42.6 (t), 41.1 (t, 2C), 19.7 (q), 13.6 (q, 2C). MS, *m/z (%)*: 233 (M^+, 3.9%); HRMS for $C_{14}H_{19}NO_2$, calcd: 233.142, found: 233.143. $[\alpha]^{20}_D$ +22.1 (*c* 2.10, $CHCl_3$, 73% ee). Enantiomeric excess determination: CP Chirasil Dex CB column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 130 °C, 30 min, 10 °C / min to 170 °C. $T_{det/inlet}$ = 250 °C, split ratio 70:1, t_{minor} = 32.70 min, t_{major} = 32.85 min, t_{SM} = 33.53 min.

1-Trimethylsilylethyl *N,N*-diethyl carbamate (2.57):



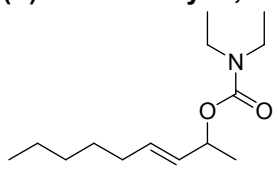
^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.55 (q, *J*= 7.8 Hz , 1H), 1.23 (d, *J*= 7.5 Hz, 3H), 1.09 (t, *J*=6.9 Hz, 6H), 0.02 (s, 9H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 156.5 (s), 64.5 (d), 41.5 (t, 2C), 16.1 (q), 13.8 (q, 2C), -4.0 (q, 3C). MS, *m/z (%)*: 217 (M^+, 1%); HRMS for $C_{10}H_{23}NO_2Si$, calcd: 217.150, found: 217.150. $[\alpha]^{20}_D$ +16.4 (*c* 1.54, $CHCl_3$, 43% ee). Enantiomeric excess determination: CP Chirasil Dex CB column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 80 °C, 20 min, 10 °C / min to 170 °C. $T_{det/inlet}$ = 250 °C, split ratio 70:1, t_{minor} = 25.08 min, t_{major} = 25.22 min, t_{SM} = 30.07 min.

Procedure for the preparation of the racemic mixtures of 2.58 and 2.59:[78]

The ketone (1 mmol) was dissolved in MeOH 2.5 mL at 0 °C and $NaBH_4$ (38 mg, 1 mmol) was added in one portion while stirring. The stirring was continued for 15-20 min., until evolution of gas ceased, after which the solvent was removed under reduced pressure. The residue was taken in ether, washed with saturated solution of NH_4Cl, water, brine and dried over Na_2SO_4. After filtration through a plug of silica gel, the solvent was removed under reduced pressure affording the desired alcohol (91-90%, respectively).

The alcohol (0.8 mmol), dry pyridine (1 mL) and *N*,*N*-diethyl carbamoyl chloride (105 μL, 0.8 mmol) were stirred at 100 °C for 4 h. The reaction mixture was poured over a slurry of ice and extracted with ether (3 x 1 mL). The combined organic layers where washed with 10% aq. HCl solution, with a saturated aq. solution of $NaHCO_3$ and dried over $MgSO_4$. Removal of the solvent under reduced pressure followed by silica gel flash chromatography (pentane / ether 9:1) afforded the desired products (86-94%, **2.58** and **2.59**, respectively).

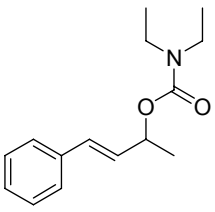
(*E*)-3-Nonen-2-yl *N,N*-diethyl carbamate (2.58):



^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.66 (dt, *J*= 15.6, 6.9 Hz, 1H), 5.45 (dd, *J*= 15.3, 6.6 Hz, 1H), 5.28-5.16 (m, 1H), 3.25 (br, 4H), 2.00 (q, *J*= 6.9 Hz, 2H), 1.42-1.16 (m, 9H), 1.09 (t, *J*= 6.9 Hz, 6H), 0.86 (t, J= 6.6 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.4 (s), 151.0 (s), 132.2 (d), 130.3 (d), 71.4 (d), 41.4 (t, 2C), 32.1 (t), 31.3 (t), 28.7 (t), 22.5

(t), 20.8 (q), 14.0 (q, 2C). MS, *m/z (%)*: 241 (M^+, 23.1%); HRMS for $C_{14}H_{27}NO_2$, calcd: 241.204, found: 241.205. $[\alpha]^{20}_D$ -4.7 (*c* 1.50, $CHCl_3$, 97% ee).
Enantiomeric excess determination: Chiraldex α-TA column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 95 °C, 150 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 200 °C, t_S = 134.1 min, t_R = 136.1 min, t_{SM} = 149.6 min. Saturated racemic product: t= 130.4, 132.0 min.

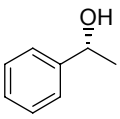
(*E*)-4-Phenyl-3-buten-2-yl *N,N*-diethyl carbamate (2.59):



^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.40-7.11 (m, 5H), 6.54 (d, *J*= 15.9, 1H), 6.18 (dd, *J*= 15.9, 6.3 Hz, 1H), 5.47-5.35 (m, 1H), 3.25 (brq, *J*= 6.6 Hz, 4H), 1.37 (d, *J*= 6.6 Hz, 3H), 1.08 (t, *J*= 6.9 Hz, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 155.3 (s), 136.6 (s), 130.5 (d), 130.0 (d), 128.5 (d, 2C), 127.6 (d), 126.5 (d, 2C), 71.2 (d), 41.3 (t, 2C), 20.7 (q), 14.0 (q, 2C). MS, *m/z (%)*: 247 (M^+, 26.5%); HRMS for $C_{15}H_{21}NO_2$, calcd: 247.157, found: 247.158.
Enantiomeric excess determination: Chiraldex α-TA column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 150 °C, 50 min, 1 °C / min to 180 °C. $T_{det/inlet}$ = 200 °C, t_S = 43.8 min, t_R = 44.8 min, t_{SM} = 70.8 min. Saturated racemic product: t= 27.5, 27.8 min.

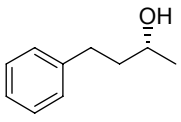
General procedure for carbamate deprotection:[79]

1-Phenyl-ethanol (2.74):



To a solution of 1-phenylethyl *N,N*-dimethyl carbamate (**2.6a**) (41.1 mg, 0.21 mmol) in ether (3 mL) at 0 °C, $LiAlH_4$ (12 mg, 0.32 mmol, 1.5 eq.) was added in one portion and the mixture was allowed to reach room temperature. After stirring for two hours the reaction was quenched with $Na_2SO_4 \cdot 10H_2O$. After filtration of the solids over celite, the solvent was removed under reduced pressure. The crude mixture was purified by silica gel flash chromatography (heptane / ethyl acetate 3:1) affording the desired compound (81%). Analytical data were in agreement with a commercial sample.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34-7.20 (m, 5H), 4.80 (q, *J*= 6.6 Hz, 1H), 2.55 (s br, 1H), 1.43 (d, *J*= 6.6 Hz, 3H).
Absolute configuration determination: β-DEX 120 column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 105 °C, $T_{det/inlet}$ = 150 °C, t_R = 27.26 min, t_S = 29.64 min.

4-Phenyl-butan-2-ol (2.75):[80]



(*E*)-1-Methyl-3-phenyl-allyl *N,N*-diethyl carbamate (**2.59**) (50 mg, 0.20 mmol) was reacted as described in the general procedure using THF as solvent. As the reaction appeared not to proceed, another equivalent of $LiAlH_4$ was added and the reaction mixture was allowed to stir at room temperature overnight and at 60 °C for two hours. After the described work-up, the crude mixture was purified by silica gel flash chromatography (pentane / ether 7:3) affording 4-phenyl-butan-2-ol (56%)

and not the expected (*E*)-4-phenyl-but-3-en-2-ol. Data were in agreement also with the product obtained from the reduction of the corresponding ketone with $NaBH_4$.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.29-7.09 (m, 5H), 3.85-3.72 (m, 1H), 2.80-2.56 (m, 2H), 1.81-1.67 (m, 2H), 1.34 (br s, 1H), 1.19 (dd, *J*= 6.2, 1.8 Hz, 3H).
The absolute configuration was determined by HPLC: Chiralpack AD column, heptane/*i*-PrOH 95:5. t_R = 8.45 min, t_S = 11.31 min.[80]

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Chapter 3
Enantioselective Rh-Catalyzed Hydrogenation of *N*-Formyl α-Dehydroamino Esters with Monodentate Phosphoramidite Ligands

Enantioselectivities up to 99% ee were achieved in the rhodium-catalyzed asymmetric hydrogenation of N-formyl α-dehydroamino esters using monodentate phosphoramidites as chiral ligands. The substrates were synthesized by condensation of methyl isocyanoacetate with a range of aldehydes and with cyclohexanone. A highly convenient multigram scale one step synthesis of methyl 2-(formamido)acrylate was developed. This compound was used in the synthesis of methyl 2-(formamido)cinnamate via a solvent-free Heck reaction. The versatility of the formyl protection was established by its removal under mild conditions.

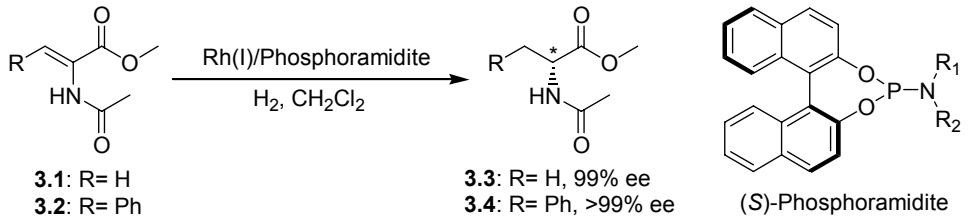
Part of this chapter has been published: Panella, L.; Marco Aleixandre, A.; Kruidhof, G. J.; Robertus, J.; Feringa, B. L.; de Vries, J. G.; Minnaard, A. J. *J. Org. Chem.* **2006**, 71, 2026.

3.1 Introduction

Enantiopure natural and unnatural α-amino acids play a fundamental role as building blocks in the preparation of many pharmaceutical and biological target compounds.[1] They are also widely used in organic chemistry as chiral auxiliaries and as catalysts.[2] Unnatural α-amino acids in particular have received increasing attention in drug discovery and protein engineering, because of novel and interesting properties they confer to biologically relevant peptides.[3] Therefore, it is not surprising that, in industry as well as in academia, there is a great interest in the use of catalytic asymmetric approaches for the preparation of optically active α-amino acids and their derivatives.[4]

Among these different approaches,[5] rhodium-catalyzed asymmetric hydrogenation is the most widely applied enantioselective chemo-catalytic technology for the synthesis of α-amino acid derivatives.[6] The reaction is extremely clean and efficient, as only substrate, solvent, hydrogen and a small amount of catalyst are needed. Since their introduction, an enormous number of bidentate chiral phosphorus ligands has been prepared,[7] as they were considered to be the key to high enantioselectivity. More recently,[8] a number of monodentate phosphorus based chiral ligands have demonstrated to be equally effective, or in some cases even superior to bidentate ligands, providing easier access to new catalysts.[9] Thus, Rh-catalyzed asymmetric hydrogenation is a well established, though still evolving[10] technology on both laboratory and industrial scale.[11]

In our laboratories, BINOL-derived monodentate phosphoramidites have been successfully used in the Rh-catalyzed hydrogenation of a series of substrates, including the benchmark substrates methyl 2-(acetamido)acrylate (**3.1**) and methyl (*Z*)-2-(acetamido)cinnamate (**3.2**) (Scheme 3.1).[12]



Scheme 3.1 *Asymmetric hydrogenation of α-dehydroamino esters (**3.1-2**) using monodentate chiral phosphoramidite ligands*

It is generally accepted that, in order to achieve excellent enantioselectivities in the Rh-catalyzed asymmetric hydrogenation of α-dehydroamino esters, the presence of the α-acetamido group coordinating to the rhodium is essential (Figure 3.1).[13]

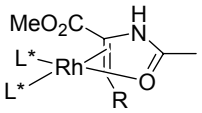


Figure 3.1 *Coordination provided by the N-acyl moiety, as stereo-directing functionality*

In an early study conducted by Glaser and coworkers, the influence of the nature of the stereo-directing group has been explored in relation to the reactivity and enantioselectivity obtained. Using DIOP (**L1**) as chiral bis-phosphine ligand the superiority of the acetamido substituent was clearly established in terms of both reactivity and enantioselectivity.[14]

3.2 α-Dehydroamino esters synthesis

α-Dehydroamino acid derivatives are important not only as substrates for asymmetric hydrogenation reactions, but they have been identified also as constituents of low molecular weight cyclic compounds from microbial sources.[15] They are precursors in the biosynthesis of some heterocyclic metabolites such as penicillin and cephalosporin (Figure 3.2).

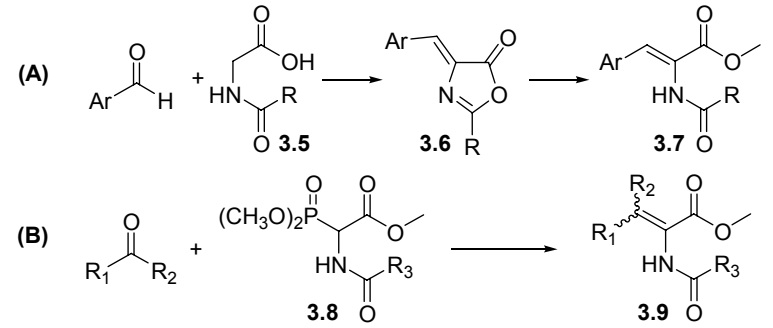
Penicillin Cephalosporin

Figure 3.2 *Structures of penicillin and cephalosporin*

Moreover, α-dehydroamino acid residues have been introduced in several bioactive peptide sequences as their presence confers increased resistance to enzymatic degradation. They are considered an important tool in the understanding of structure-function relationship in biologically active peptides, as demonstrated by the dehydro analogues of some of the peptide hormones such as angiotensin and gramicidin S.

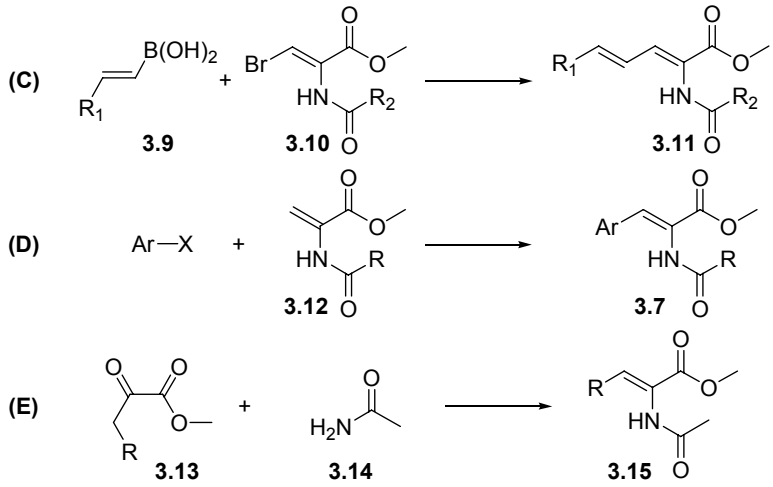
There is extensive literature on the preparation of α-dehydroamino acid derivatives and frequently used methods are depicted in Scheme 3.2 and Scheme 3.3.[16] The Erlenmeyer synthesis via azlactone **3.6**[17] (Scheme 3.2, method A) and the condensation of aldehydes with phosphorylglycine esters **3.8** (method B) are the most frequently used.[18] The main limitation of the azlactone approach lies in the harsh reaction conditions, which limits its use to aromatic aldehydes devoid of acid sensitive groups.[19] In addition, substrates with carbamate protecting groups cannot be prepared. The use of phosphorylglycine esters **3.8** is instead more versatile and the synthesis is milder. This method is complementary to the Erlenmeyer synthesis

as it allows access to β-alkyl α-dehydroamino acids derivatives. Nevertheless, although also ketones can be used, reaction times in this case are long.[20] Moreover, the preparation of the Horner-Emmons reagent requires several steps.



Scheme 3.2 *Synthesis of* α*-dehydroamino esters via: (A) Erlenmeyer azlactone synthesis. (B) Horner-Emmons method with Schmidt's phosphorylglycine esters **3.8***

Both β-aryl (**3.7**) and β-alkenyl (**3.11**) substituted α-dehydroamino acids derivatives can be conveniently obtained through Suzuki coupling (Scheme 3.3, method C).[21] Nevertheless, also in this case the preparation of the starting methyl β-bromo-(acetamido)acrylate **3.10** is quite laborious and low yielding. Arylation of methyl 2-(acylamido)acrylates **3.12** is achieved using the Heck reaction (method D).[22] This method, despite its potential, is somewhat neglected, once more because of the problematic synthesis of the starting material, especially on large scale.[23]



Scheme 3.3 *Synthesis of* α*-dehydroamino esters via: (C) Suzuki cross-coupling. (D) Heck arylation. (E) Acetamide-α-ketoester condensation.*

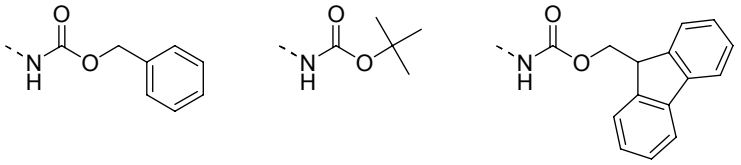
The condensation of α-ketoesters **3.13** and acetamide (**3.14**) (method E), allows, in principal, the preparation of a large variety of α-enamides. However, also in this case, its use is limited by the harsh reaction conditions and low yields.[16]

3.3 Amine protecting groups

Ultimately, the driving force for the preparation of enantiopure α-amino acid derivatives is their use. In this respect, the ease of their modification and manipulation plays an important role. The chemistry of α-amino acids evolved around the development of suitable protection and deprotection procedures of their functional groups. Therefore, the usefulness of a protecting group is generally measured in its ability to fulfill requirements such as:[24]

- Cleavage must proceed without compromising the stability of other temporary protecting groups or peptide bonds.
- No racemization must occur during its introduction or removal.
- Solubility of the protected α-amino acid derivatives must be favorable.

A huge number of amine protecting groups have been developed and they are generally classified by their cleavage conditions: acidolysis, base cleavage, reduction/oxidation, nucleophilic substitution, photolysis and enzymatic hydrolysis. In practical terms, the use of the base-labile 9-fluorenylmethoxycarbonyl group (Fmoc) and deprotection under moderately acidic conditions, due to the stability of the peptide bond (Z, Boc), are usually preferred. In Figure 3.3 the most commonly used amino protecting groups and their deprotection conditions are depicted.



Cbz (**Z**):
H_2/Pd, HBr/AcOH,
Na/liq. NH_3

Boc:
TFA, TFA/CH_2Cl_2,
HCl/organic solvents

Fmoc:
piperidine, DBU,
2-aminoethanol,
morpholine, liq. NH_3

Figure 3.3 *Prominent urethane-type amino protecting groups*

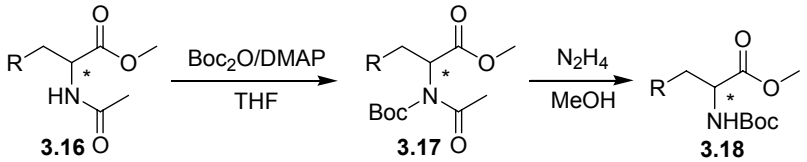
More recently also carbamate stereo-directing groups (Boc and Z) have shown to be effective in asymmetric hydrogenation reactions,[25] the acyl moiety (Figure 3.4) is still the most employed stereo-directing group. Nevertheless, it is generally not used as protecting group in peptide synthesis due to the similarity of the resulting acylamide group to the peptide bond, the easy racemization of activated α-amino acid derivatives possessing a *N*-acyl protection and the use of strongly acidic conditions (1.2 N HCl, reflux, 9h)[26] needed for its removal.[27] This might cause degradation of sensitive functionalities or loss of optical purity. Examples of

racemization occurring during deprotection under acidic conditions have been reported also for *N*-benzoyl (Figure 3.4) functionalized products.[28]

Acetamide Benzamide

Figure 3.4 *Acyl protections generally used in asymmetric hydrogenation reactions*

Deprotection using basic conditions is precluded because of competing azlactone (**3.6**) formation with subsequent racemization.[29] To overcome these problems, Burk and coworkers envisioned a milder deprotection by first converting the acylamide **3.16** in its Boc derivative **3.17**, followed by deacetylation and Boc removal, respectively.[30]



Scheme 3.4 *Milder acyl deprotection by Boc replacement*

Nevertheless, this implies an extra transformation. Mild deprotection is also provided by enzymatic hydrolysis using acylases, but acylases that can hydrolyze the unnatural *R*-enantiomer are not commercially available.[31]

3.4 Scope of this study

Whilst the asymmetric hydrogenation step has been developed very well, both the synthesis of the substrates and the removal of the directing group on the amine after hydrogenation limit the scope and applicability of this strategy.

Clearly, there is a need for:

- A more general, mild and easily applicable synthetic method for the preparation of the required α-dehydroamino esters.
- A directing group that can be removed under mild conditions.
- A suitable protecting group that can be used directly for other transformations, such as, for example, peptide synthesis.

We envisaged that all these requirements can be met using a formyl group as stereo-directing functionality (Figure 3.5).

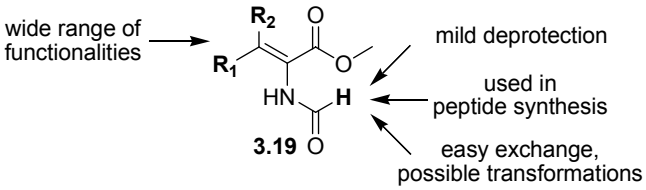
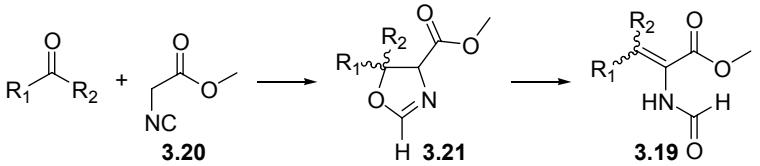


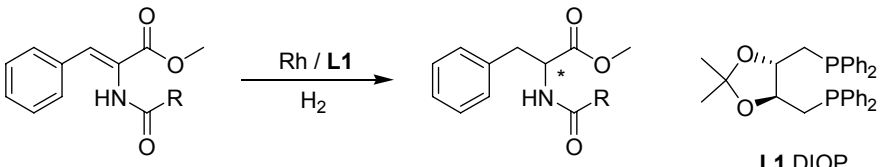
Figure 3.5 *Advantages in the use of a formamido moiety as stereo-directing group*

The formyl moiety, as protective group, is considered a desirable choice due to its low cost, easy introduction and removal and improved water solubility.[32] For the synthesis of the required 2-(formamido)acrylate derivatives **3.19**, we adopted the Schöllkopf method involving the condensation of aldehydes and ketones with methyl isocyanoacetate (**3.20**).[33] The ring opening by elimination of the 2-oxazolines **3.21** yields the desired *N*-formyl α-dehydroamino esters **3.19** (Scheme 3.5).



Scheme 3.5 *Synthesis of N-formyl protected* α*-dehydroamino esters **3.19** via the Schöllkopf 2-oxazolines **3.21***

The main reason why the formyl group is not used in asymmetric hydrogenation seems directly connected to the results obtained by Glaser and coworkers (Scheme 3.6).[14] The direct comparison between the Rh-catalyzed asymmetric hydrogenation of *N*-acyl (**3.2**) and *N*-formyl (**3.22**) cinnamic acid methyl ester using DIOP (**L1**) as chiral ligand showed a considerable decrease in reactivity and enantioselectivity in the latter case (69% and 58% ee, respectively).



3.2: R= CH_3
3.22: R= H

3.4: R= CH_3, 69% ee
3.23: R= H, 58% ee

Scheme 3.6 *Methyl (Z)-2-(acetamido)cinnamate (**3.2**) vs. methyl (Z)-2-(formamido)cinnamate (**3.22**) using a Rh-DIOP (**L1**) catalyst*

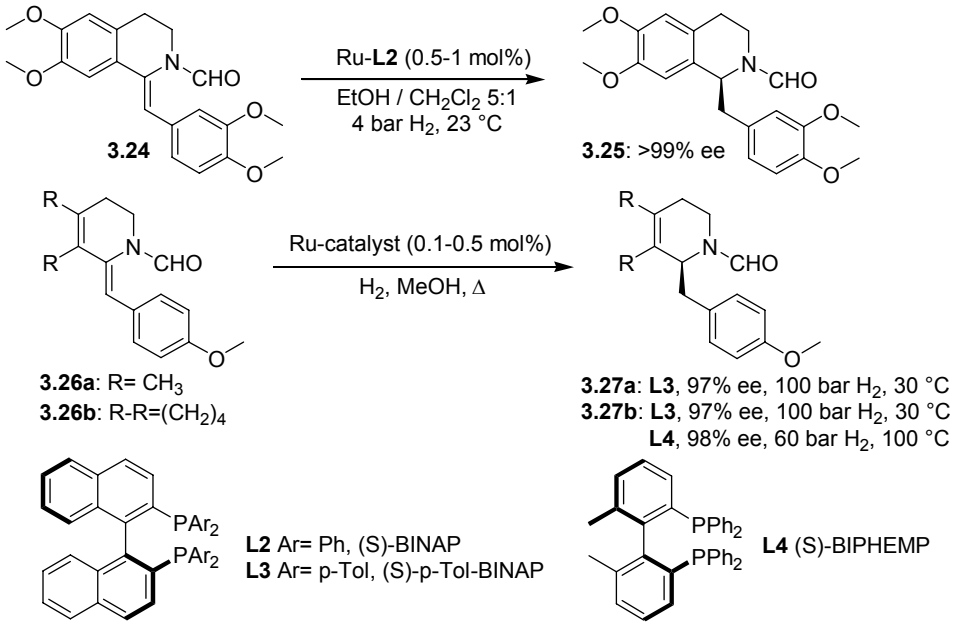
Not surprisingly, the conclusion drawn from these studies was that an acyl moiety coordinates stronger to the metal center than a formyl group. The deviation observed was attributed to both electronic and steric effects, the methyl group being bigger than a proton and more strongly electron donating in nature. In

addition, from a synthetic point of view, *N*-formyl α-dehydroamino esters **3.19** cannot be prepared using the Erlenmeyer azlactone synthesis. Indeed, when *N*-formyl glycine was used in the Erlenmeyer azlactone synthesis with benzaldehyde, the corresponding methyl substituted azlactone **3.6** was isolated in 70% yield, due to the in situ acylation-deformylation of the substrate.[34]

In view of the fact that catalysts have evolved tremendously since the introduction of DIOP (**L1**), we felt the use of formyl as stereo-directing group should be reconsidered. Therefore, this study focused on the practical preparation of *N*-formyl α-dehydroamino esters **3.19** and their enantioselective hydrogenation, using Rh-phosphoramidite catalysts.

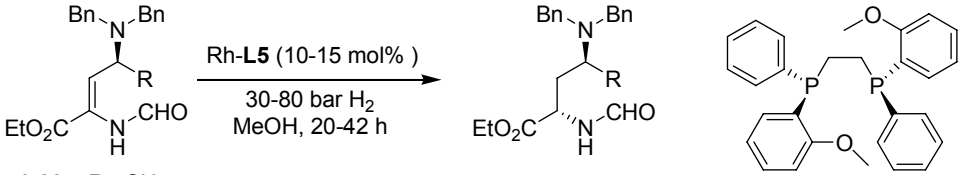
3.5 Precedents of the *N*-formyl as stereo-directing group

Surprisingly, the formyl moiety as stereo-directing group is rarely used in Rh-catalyzed enantioselective hydrogenation. Cyclic *N*-formyl enamides **3.24** and **3.26a-b** (Scheme 3.7) have been hydrogenated first by Noyori[35] and subsequently by Heiser[36] and their coworkers using Ru-BINAP (**L2**) and Ru-BIPHEMP (**L4**), respectively. The compounds **3.25** and **3.27a-b** obtained with high enantioselectivities were key intermediates in the synthesis of morphine analogues.



Scheme 3.7 *Use of the formyl moiety in substrates for Ru-catalyzed asymmetric hydrogenation*

Reetz and Kayser reported the diastereoselective Rh-catalyzed asymmetric hydrogenation of a range of enantiopure γ-amino *N*-formyl α-dehydroamino esters **3.28a-b** employing DIOP (**L1**), DIPAMP (**L5**) and BINAP (**L2**) as chiral ligands.[37] The best results were obtained using DIPAMP (**L5**) and they are depicted in Scheme 3.8.



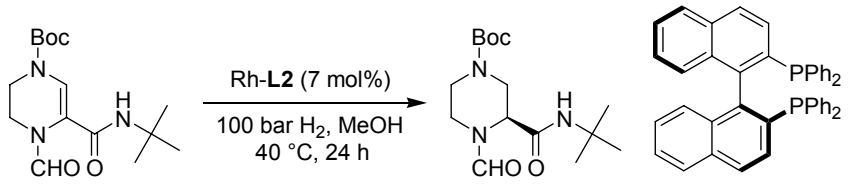
3.28a: R= CH_3
3.28b: R= CH_2OH

3.29a: d.r.= 98:2
3.29b: d.r.= 97:3

L5 (R,R)-DIPAMP

Scheme 3.8 *Diastereoselective Rh-catalyzed asymmetric hydrogenation of enantiopure γ-amino N-formyl* α*-dehydroamino esters **3.28a-b***

An isolated case is the Rh-catalyzed enantioselective hydrogenation of an *N*-formyl, *N*-Boc protected tetrahydropyrazine (**3.30**, Scheme 3.7) reported by Rossen and coworkers.[38] However, in this case it is unclear whether the directing group is the formyl or the Boc moiety.



3.30

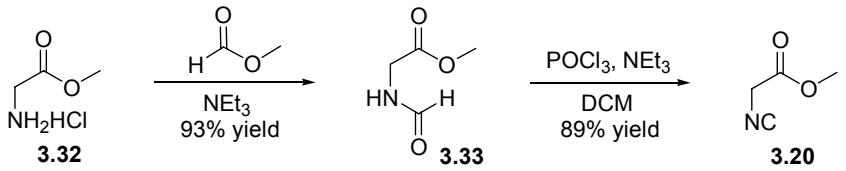
3.31: 97% ee

L2 (S)-BINAP

Scheme 3.9 *Rh-catalyzed enantioselective hydrogenation of substrates bearing a formamido moiety*

3.6 Synthesis of *N*-formyl α-dehydroamino esters

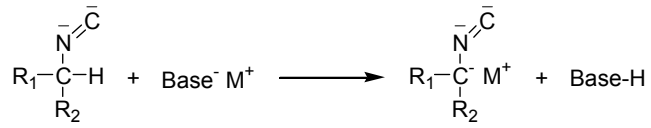
N-formyl α-dehydroamino esters **3.19** can be obtained by mild aldol condensation of methyl isocyanoacetate (**3.20**) with a variety of alkyl, aryl, or heterocyclic aldehydes and ketones.[16a] Methyl isocyanoacetate (**3.20**) is commercially available and can be conveniently prepared, in good yields and on a multigram scale, via *N*-formylation of glycine methyl ester (**3.32**) followed by subsequent dehydration using $POCl_3$ (Scheme 3.10).[39]



3.32 **3.33** **3.20**

Scheme 3.10 *Synthesis of methyl isocyanoacetate (**3.20**)*

The usefulness of **3.20** has been proven in different occasions since Schöllkopf and Gerhart achieved the α-metalation of isocyanides **3.34** using alkaline metal based reagents (Scheme 3.11).[40]

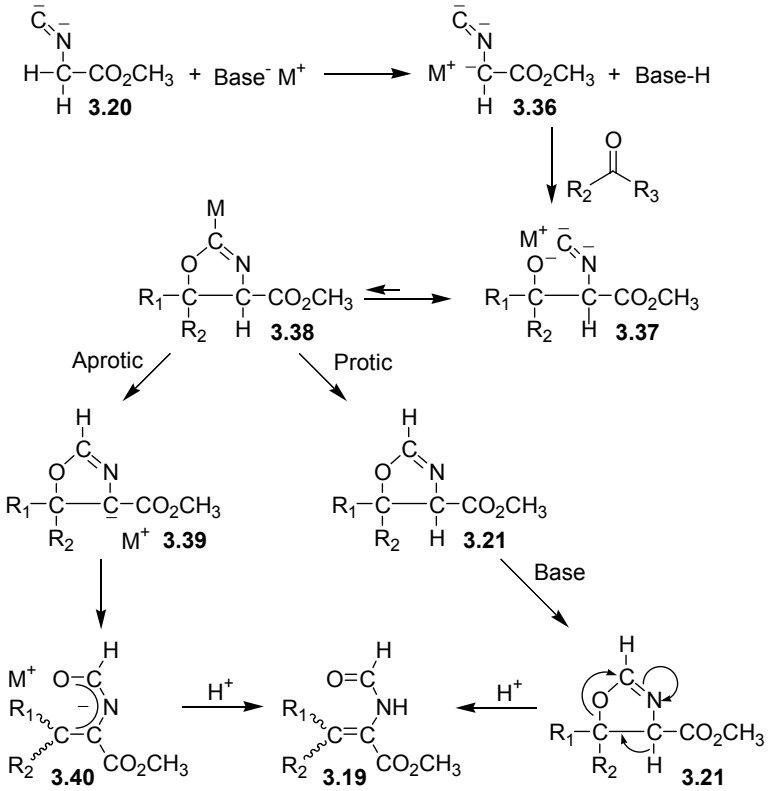


3.34 **3.35**

M= Li, Na, K

Scheme 3.11 *Activating effect of an isocyano group: easy α-metalation of isocyanides **3.34***

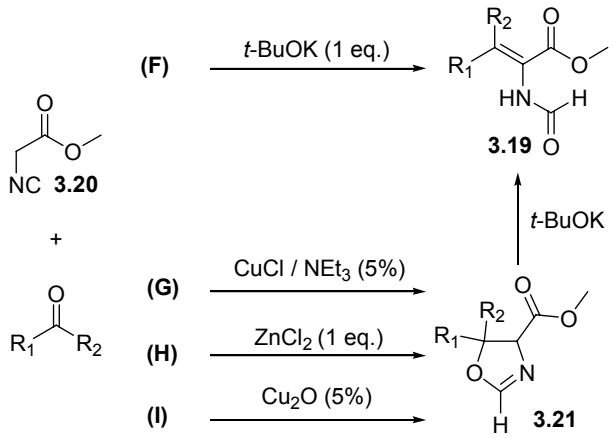
As shown in Scheme 3.12, the α-metalated intermediate **3.36** of methyl isocyanoacetate can be reacted with compounds containing polar double bonds such as aldehydes and ketones affording the carbonyl adduct **3.37** and the isomeric 2-metalated **3.38**, which in the presence of protic solvents (for example MeOH) affords the desired 2-oxazolines **3.21**. When treated with base and upon acidic work-up, 2-oxazolines **3.21** undergo ring opening yielding methyl 2-(formamido)acrylate derivatives **3.19**.[41] Instead, hydrolysis in conc. HCl and protic solvents affords amino alcohols. Methyl 2-(formamido)acrylate derivatives **3.19** are obtained directly from the rearrangement of **3.38** into **3.40** *via* **3.39** when performing the reaction in aprotic solvents (such as THF) followed by acidic work-up.[42]



Scheme 3.12 *Synthesis of 2-oxazolines **3.21** and the corresponding N-formyl* α*-dehydroamino esters **3.19***

Therefore, *N*-formyl α-dehydroamino esters **3.19** can be prepared either in a single step, by performing the condensation in the presence of *t*-BuOK (Scheme 3.13, method F), or *via* the isolation and subsequent ring opening of the corresponding 2-oxazolines **3.21**.[43]

Ito and coworkers introduced the use of the Lewis acids CuCl and $ZnCl_2$ for the synthesis of 2-oxazolines **3.21** (methods G and H).[44] The use of Cu_2O proved to be beneficial when using ketones in the condensation reaction instead of aldehydes (method I).[45] Subsequent treatment of the 2-oxazolines **3.21** with *t*-BuOK would lead to the α-dehydroamino esters **3.19**. It was decided to compare the various methods present in the literature for the preparation of 2-oxazolines **3.21** and develop a one pot synthesis of *N*-formyl α-dehydroamino esters **3.19** that would avoid their isolation.



Scheme 3.13 *Synthetic approaches for the preparation of N-formyl* α*-dehydroamino esters **3.19***

The substrates shown in Figure 3.6 were prepared adopting the different methods depicted in Scheme 3.13 and the results are shown in Table 3.1.

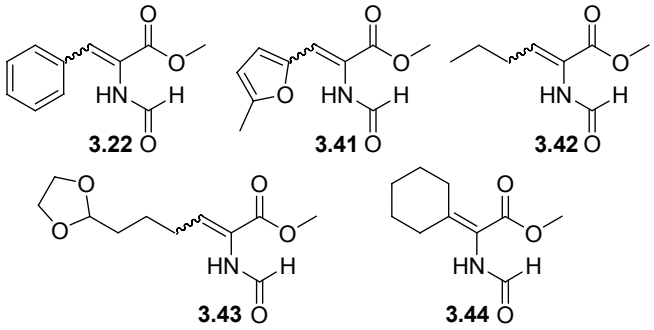
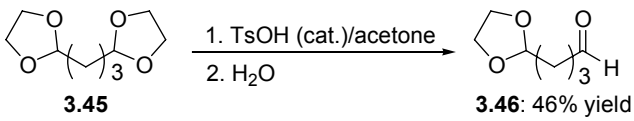


Figure 3.6 *Substrates prepared for this study*

Aldehyde 5-(1,3-dioxolan-2-yl)-1-pentanal (**3.46**), used as starting material for the synthesis of substrate **3.43,** was not commercially available. Therefore, it was obtained by monodeprotection of the corresponding bis-1,3-dioxolane (**3.45**), adapting a literature procedure reported by Takacs and coworkers.[46]

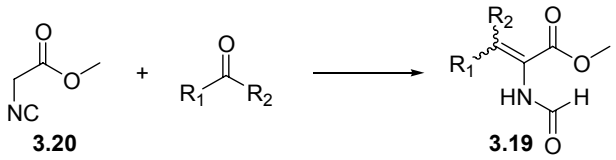


Scheme 3.14 *Synthesis of 5-(1,3-dioxolan-2-yl)-1-pentanal (**3.46**)*

The sole use of acetone and a catalytic amount of *p*-toluenesulfonic acid originally employed proved to be problematic, as the product was obtained only in trace

amounts. The addition of water to the reaction mixture after 30 minutes proved to be critical and was followed by stirring for an additional 30 minutes. The desired aldehyde **3.46** was isolated upon distillation in 46% yield.

Table 3.1 *Synthesis of N-formyl α-dehydroamino esters **3.19** with methyl isocyanoacetate (**3.20**)*

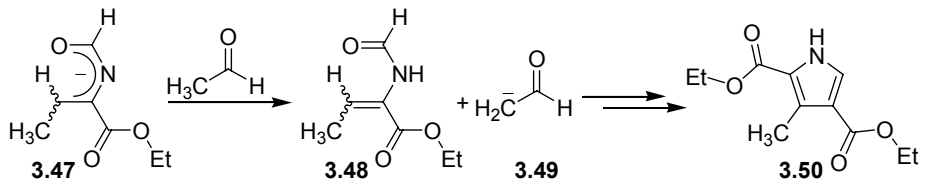


3.20 **3.19**

entry	product	method[a]	Z:*E*	yield (%)
1	**3.22**	I	2.9:1	83
2	**3.41**	F	5.7:1	30
3	**3.41**	G	1.9:1	79
4	**3.42**	F	1.2:1	29
5	**3.42**	G	1.5:1	63
6	**3.42**	I	1.6:1	65
7	**3.43**	G	1.5:1	58
8	**3.44**	H	-	35
9	**3.44**	I	-	82

[a]Method F: (a) 1 eq *t*-BuOK, THF, -60 °C; (b) 1 eq AcOH, CH_2Cl_2, 0 °C. Method G: (a) CuCl/NEt_3 (5%), THF, rt; (b) 1 eq *t*-BuOK THF, 0 °C; (c) 1 eq AcOH, CH_2Cl_2, 0 °C. Method H: 1 eq $ZnCl_2$, THF, rt; (b) 1 eq *t*-BuOK THF, 0 °C; (c) 1 eq AcOH, CH_2Cl_2, 0 °C. Method I: (a) Cu_2O (5%), Et_2O, rt; (b) 1 eq *t*-BuOK THF, 0 °C; (c) 1 eq AcOH, CH_2Cl_2, 0 °C.

The direct use of *t*-BuOK in the condensation reaction (Scheme 3.13, method F) proved to be less efficient (Table 3.1, entries 2 and 4) as the products **3.41** and **3.42** were obtained in 30% and 29% yield, respectively, from complex crude mixtures. A reason for the poor outcome of these two condensation reactions could be found in the acidity of the α-protons present on the aldehydes used, as also postulated by Schöllkopf and coworkers when using BuLi and acetaldehyde (Scheme 3.15).[47]



3.47 **3.48** **3.49** **3.50**

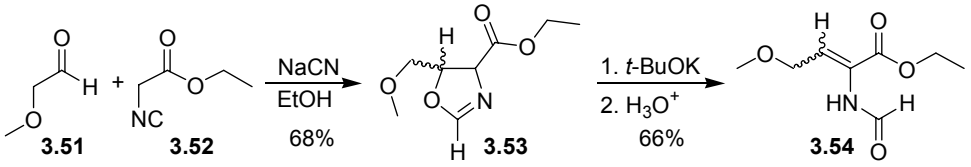
Scheme 3.15 *Aldehydes with α-protons as source of side reactions*

The abstraction of the α-proton from acetaldehyde (**3.49**) was supposed to be the starting point for another series of side reactions ending in the formation of pyrroles **3.50**. Method F is also known to be sensitive to the quality of *t*-BuOK used, which was therefore freshly sublimed prior to use.[42a] Moreover, this approach seemed to suffer in reproducibility as methyl 2-(formamido)cinnamate (**3.22**) prepared using *t*-BuOK failed to reach the reported yield (51% vs. 85%).[48] For these reasons, this method seemed to be less practical and general.

On the other hand, products **3.41** and **3.42** could be obtained in good yields by using the stepwise approach of method G (entries 3 and 5). The reactions were run overnight at room temperature in the presence of a catalytic amount of CuCl and NEt_3 (5%). In most cases, ^{1}H-NMR of the crude mixtures showed complete conversion to the 2-oxazoline intermediate **3.21**. In all cases using Cu(I) or Zn(II) the 2-oxazolines **3.21** were converted in situ to the desired *N*-formyl protected α-dehydroamino esters **3.19** by adding *t*-BuOK in THF to the reaction mixture, followed by acetic acid in CH_2Cl_2. The use of Cu(I) also allowed a satisfactory synthesis of the more delicate substrate **3.43** (entry 7).

Aldol condensation with methyl isocyanoacetate **3.20** using ketones as electrophiles has not been frequently described in literature. Ito and coworkers described the use of $ZnCl_2$ (1 eq.) in combination with 2-cyclohexenone yielding the corresponding 2-oxazoline in 29% yield.[44] Applying the same methodology, α-dehydroamino ester **3.44** was obtained in 35% yield (entry 8).[49] However, a very good 82% yield was obtained using a catalytic amount of Cu_2O (entry 9). The same procedure was adopted using benzaldehyde for the synthesis of **3.22**, obtained in 83% yield (entry 1).[50] Furthermore, Cu_2O was also used for the preparation of **3.42** and the result was similar to that obtained when using CuCl (entry 6).

The use of Lewis acids requires that in the last step of the synthesis the 2-oxazolines **3.21** obtained were converted into *N*-formyl α-dehydroamino esters **3.19** in the presence of base. Hoppe and Schöllkopf reported an example in which the isolated 2-oxazoline **3.53** was treated with *t*-BuOK in THF and worked up with acetic acid in water, after the organic solvent was completely removed (Scheme 3.16).[43] An overall yield for **3.54** of 45% was obtained.



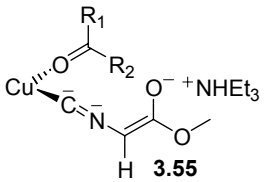
Scheme 3.16 *Ring opening of isolated 2-oxazoline **3.54***

It was expected that performing the ring opening in situ would have increased the practicality of the synthetic scheme avoiding an extra purification step. It was also decided to avoid the removal of the organic solvent and acetic acid was directly

added from a CH_2Cl_2 solution. The in situ transformation resulted to be successful, more practical and afforded good isolated yields over two steps (Table 3.1).

3.7 Some insight into the reaction mechanism using Lewis acids

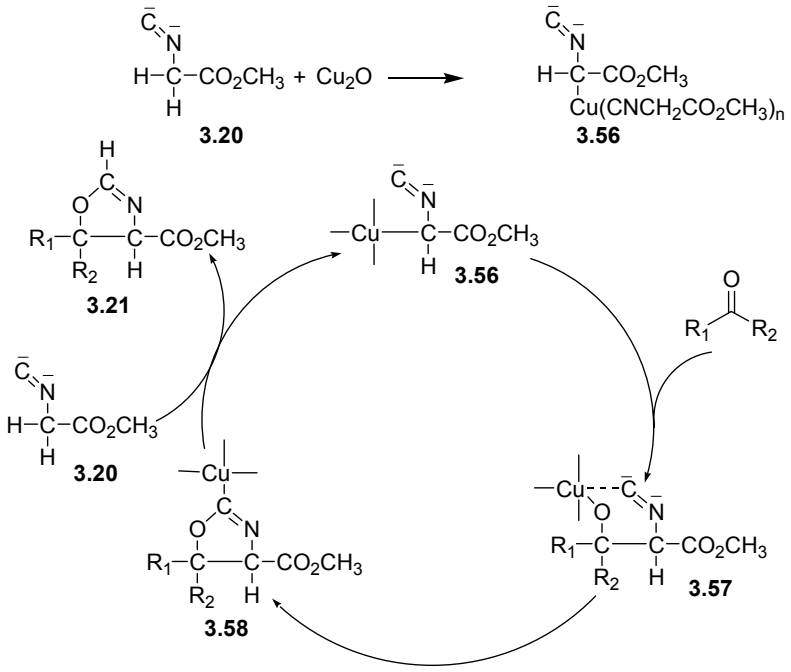
The coordination of methyl isocyanoacetate **3.20** with transition metals like Cu or Zn is assumed to increase the acidity of its α-protons allowing milder reaction conditions, cleaner reaction mixtures and better conversions to 2-oxazolines **3.21**.[45b] Ito and coworkers proposed the coordination of both methyl isocyanoacetate **3.20** and carbonyl reagent to the Lewis acid (in that case Au) postulating that the formation of the enolate **3.55** was promoted by the base.[51] This assumption was supported by the fact that condensations performed in the presence of 5 mol% of Cu(I) and NEt_3 resulted to be faster than those in which 1 equivalent of Zn(II) was used (Figure 3.7).[44]



3.55

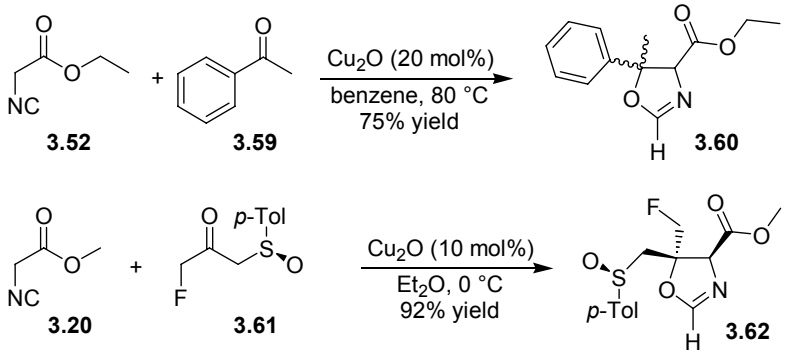
Figure 3.7 *Possible coordination of the Lewis acid in the presence of base*

Nevertheless, this does not explain the fast and high yielding reactions obtained when using a catalytic amount of Cu_2O, for which no base is used. Some insight into the mechanism of the reaction between isocyanide **3.20** and carbonyl compounds catalyzed by Cu_2O was presented by Saegusa and coworkers.[45a] The key intermediate of the cycloaddition was assumed to be an organocopper-isocyanide adduct **3.56**, in which the α-hydrogen was replaced by Cu(I) having other isocyanide molecules as ligands (Scheme 3.17). The addition of **3.56** to the carbonyl reagent would generate a copper-alkoxide complex **3.57** which would convert into the cyclized copper species **3.58**. This copper complex would then abstract the α-proton from another molecule of **3.20** yielding the desired 2-oxazoline **3.21**.



Scheme 3.17 *Proposed reaction mechanism in the presence of Cu_2O*

This would explain why the reaction proceeds in the absence of bases and protic solvents and it can be performed in the presence of a catalytic amount of Lewis acid. One of the examples reported in the original work,[45a] 2-oxazoline **3.60** was obtained in 75% yield using 20 mol% of Cu_2O and an access of carbonyl reagent **3.59** (2 eq.) performing the reaction at 80 °C and using benzene as solvent (Scheme 3.18).



Scheme 3.18 *Reaction conditions reported in literature using Cu_2O*

More recent research, reported by Resnati and coworkers,[45c] showed that it was possible to perform the reaction with a lower amount of Cu(I) (10 mol%), at lower temperature (0 °C) in ether, although only one specific ketone (**3.61**) was used.

In this investigation, as shown in Table 3.1, aldehydes and a ketone were reacted with methyl isocyanoacetate (**3.20**) in the presence of 5 mol% of Cu_2O at room temperature, broadening the potential of this synthetic pathway. In conclusion, the preferred and most versatile procedures involve the use of catalytic Cu(I), with the subsequent in situ ring opening of the 2-oxazolines **3.21** formed to yield the desired *N*-formyl α-dehydroamino esters **3.19**.

3.8 Isomers and rotamers

In all cases a mixture of *Z* and *E* alkene geometry isomers was obtained (Table 3.1, entries 1-7). The isomers were separated by flash chromatography, as we were interested in the influence of the double bond configuration on the activity and enantioselectivity in the Rh-catalyzed hydrogenation reactions. In addition, every isomer consisted of a mixture of *trans* and *cis* rotamers corresponding to slow isomerization of the formamide bond. The definition of *trans* and *cis* conformers adopts the definition of the conformation of the amide bond in a peptide backbone as depicted in Figure 3.8.[52]

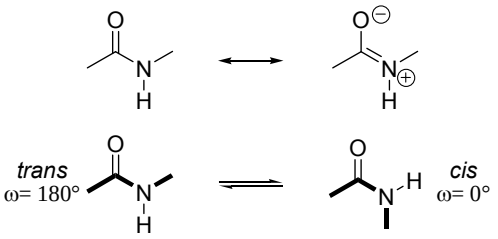


Figure 3.8 *Amide bond formalism used to define trans and cis rotamers*

Identification of the different isomers and rotamers was achieved by ^{1}H-NMR based on previous studies.[53] In order to identify *Z* and *E* isomers, the position of the vinylic H_β and of the proton on the nitrogen was very diagnostic. In the case of the *Z* isomer both signals appear at higher field compared to the corresponding *E* isomer (Figure 3.9 and Figure 3.10). In both *Z* and *E* isomers, the proton on the formyl group appears as a singlet for the *trans* rotamer and as a doublet for the *cis* rotamer. Not only the position of the formyl and the amido protons differs between *trans* and *cis* rotamers, but also the H_β vinylic proton. This facilitates further identification of the *Z* and *E* isomers, as the *trans* and *cis* H_β vinylic protons of the *E* isomer appear at clearly different chemical shifts.

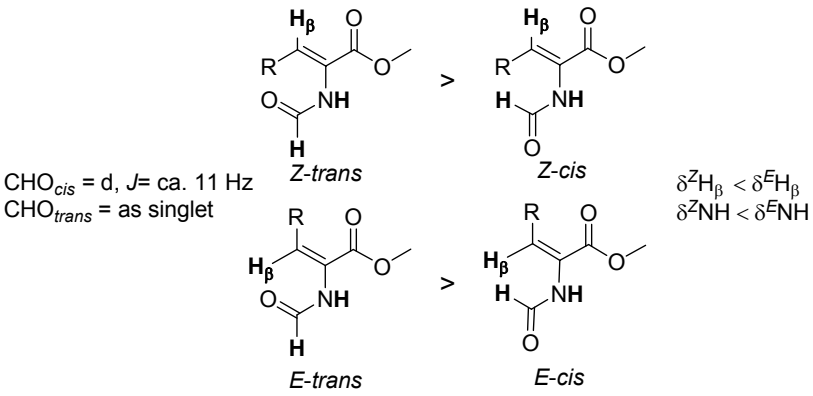


Figure 3.9 *Z and E isomers and trans and cis rotamers observed for N-formyl* α*-dehydroamino methyl esters* ***3.19***

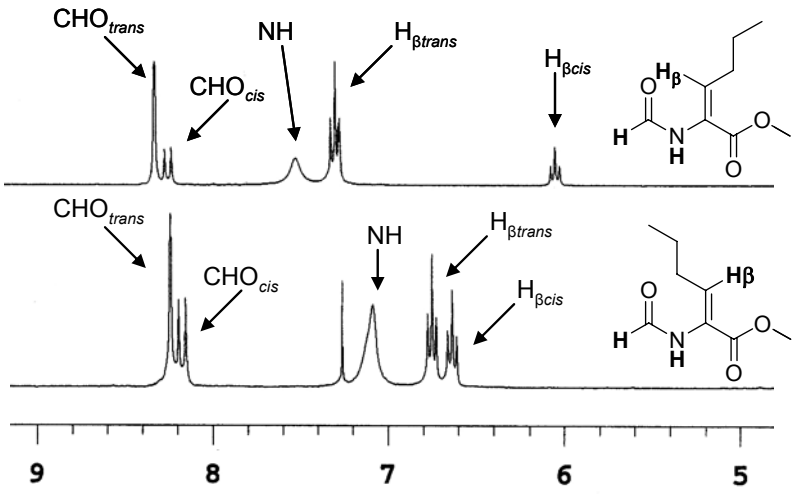


Figure 3.10 *Portion of* ^{1}*H-NMR of* ***3.42*** *(E) and* ***3.42*** *(Z) with rotamers in evidence*

For both *Z* and *E* isomers the *trans* rotamer is present as the major constituent. The ratio decreases with the increasing bulkiness of the β-substituent. For example, in the case of the products **3.22** (*Z*) and **3.41** (*Z*) the *cis* rotamer is present in major amount due to steric hindrance (Table 3.2).

Table 3.2 *Ratio of trans and cis rotamers for Z and E isomers*

entry	compound	*trans*:*cis*
1	**3.22** (*E*)	75:25
2	**3.22** (*Z*)	**47:53**
3	**3.41** (*E*)	71:29
4	**3.41** (*Z*)	**28:72**
5	**3.42** (*E*)	80:20
6	**3.42** (*Z*)	58:42
7	**3.43** (*E*)	72:28
8	**3.43** (*Z*)	58:42
9	**3.44**	65:35

The difference in chemical shift between the ^{1}H-NMR signals for the *trans* and *cis* rotamers of the methyl ester of methyl (*Z*)-2-(formamido)cinnamate (**3.22**) was conveniently used to perform a variable temperature ^{1}H-NMR experiment, which allowed to determine a coalescence temperature of T_c= 45 °C (Figure 3.11).

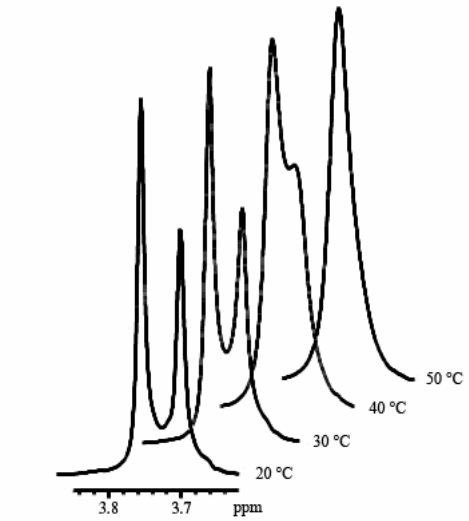


Figure 3.11 *^{1}H-NMR determination of the rotamers coalescence temperature*

From this temperature, the Gibbs energy of activation, which corresponds to the rotational barrier, was calculated to be $\Delta G^{\#}$= 63.3 kJ/mol using the equation depicted below (Eq. 3.1), where $|\nu_a-\nu_b|$ is the distance between the two signals (120 Hz).[54]

$$\Delta G^{\#} = RT_c \cdot \ln \frac{RT_c\sqrt{2}}{\pi \cdot N_A \cdot h\left|\nu_A - \nu_B\right|}$$

Equation 3.1 *Relation between rotational barrier and coalescence temperature*

Once the rotational barrier was known, a frequency of rotation at room temperature of 50 sec^{-1} (180,000 h^{-1}) was calculated using the Eyring equation (Eq. 2).[55]

$$k = \frac{RT}{N_A \cdot h} e^{-\frac{\Delta G^{\#}}{RT}}$$

Equation 3.2 *Determination of the rotational frequency*

As previously mentioned (page 86), in asymmetric hydrogenation reactions the bidentate coordination of the substrate to the rhodium complex is considered fundamental in order to obtain high enantioselectivities. As shown in Figure 3.12, the *trans* rotamer of the substrate can easily coordinate with both the double bond and the carbonyl group to the metal center allowing the desired three-points coordination.

trans rotamer *cis* rotamer

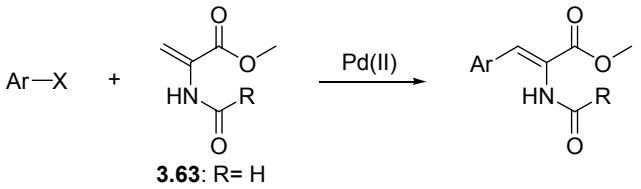
Figure 3.12 *Substrate coordination to the metal center for cis and trans rotamers*

This coordination mode is instead prevented for the *cis* rotamer. The substrate in this case would be able to coordinate only with the double bond affording a different complex which is considered to be less enantioselective.[13] Moreover, in case of instability of this alternative complex, there might be possible Rh(0) formation, with consequent lower reactivity and enantioselectivity. However, due to the fast rotation under ambient conditions, no influence of the interconversion between *cis* and *trans* rotamers was expected on the hydrogenation reactions outcome.

3.9 Preparation and use of methyl 2-(formamido)acrylate (3.63)

As a second versatile method for the preparation of *N*-formyl α-dehydroamino esters, the Heck reaction between aryl halides and methyl 2-(formamido)acrylate (**3.63**) was explored (Scheme 3.19). This approach is analogous to the one reported, among others, by RajanBabu and coworkers for the *N*-acyl equivalent

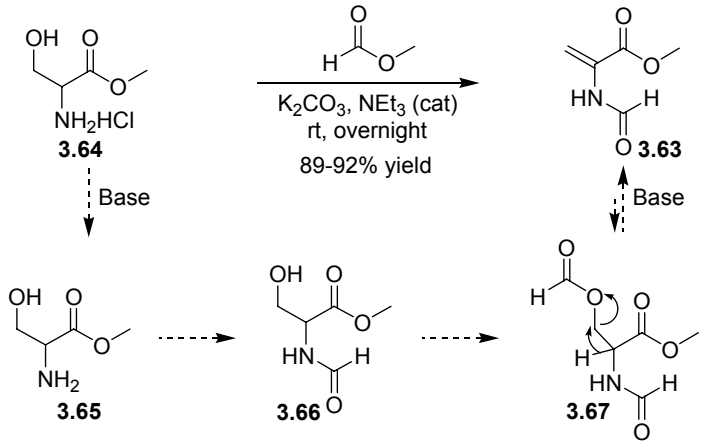
3.1.[56] This methodology gives access in two steps to numerous substrates for asymmetric hydrogenation.



Scheme 3.19 *Heck arylation reaction*

3.9.1 Synthesis of methyl 2-(formamido)acrylate (3.63)

Methyl 2-(formamido)acrylate (**3.63**) would appear to be an extremely valuable starting material, provided the possibility of establishing a cheap, practical and efficient preparation. The approach adopted is shown in Scheme 3.20. Product **3.63** was obtained by formylation and dehydration of inexpensive and readily available racemic serine methyl ester hydrochloric salt (**3.64**), in one step on a multigram scale using methyl formate as reagent and solvent.



Scheme 3.20 *Synthesis of 2-(formamido)acrylic methyl ester (**3.63**)*

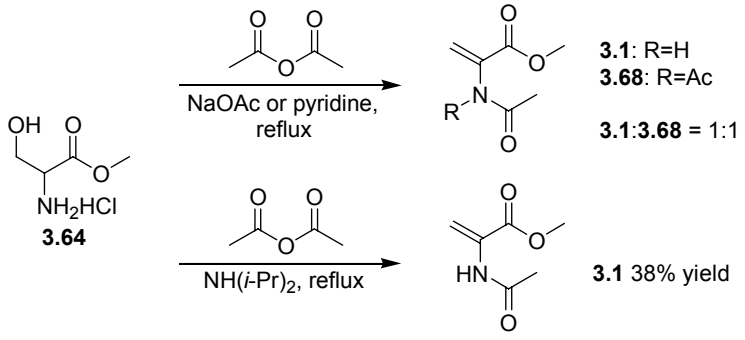
The use of K_2CO_3 (4 eq.) turned out to be essential as the salts formed were not soluble in the reaction mixture and, next to driving the reaction to completion, could be easily removed by filtration. The use of NEt_3 (100 µL on a 10 gram scale) ensured enough base in solution to keep the reaction going.

When only NEt_3 was used, the reaction was much slower and did not go to completion even after a long reaction time. This was attributed to the establishment of an equilibrium between **3.67** and **3.63** (Scheme 3.20) caused by the addition of formic acid obtained during the elimination step. Therefore, it was postulated that

removing the formate salt from the reaction mixture would prevent the equilibrium to take place. No information on the solubility of formate salts in methyl formate was found, nevertheless, ammonium formate is reported to be soluble both in alcohol and ether, whether potassium formate is soluble in alcohol but insoluble in ether.[57] The difference of solubility of the two salts in ether seemed to be a good indication and encouraged us to use K_2CO_3. This assumption turned out to be correct. The use of Cs_2CO_3, under the same reaction conditions, resulted in a faster reaction, but the product **3.63** was obtained in lower yield as a polymerization process started to take place. Alternatively, a catalytic amount of Cs_2CO_3 was added almost at the end of the reaction, resulting in the quick and complete disappearance of remaining intermediates. Nevertheless, the reaction mixture turned yellow and the yield of the reaction was lower (85% yield), again because of competing polymerization processes.

Under optimized reaction conditions, the product **3.63** was obtained in 92% isolated yield starting from 1 gram of racemic serine hydrochloric acid methyl ester (**3.64**) and in 89% starting from 10 gram. Possible causes for the slightly lower yield, while scaling up the reaction, were attributed to the not optimized amount of NEt_3 added and the efficiency of the separation of the product from the salts during filtration. Methyl 2-(formamido)acrylate (**3.63**) obtained by simple filtration is an off-white solid, which, even upon storage at 4 °C, on a long term turned into a yellow and semitransparent material with rubber consistency. Quick flash chromatography of the crude mixture, after removal of volatiles, afforded instead a white and stable compound even at room temperature. The nature of the possible impurity has not been established.

This protocol allows to obtain **3.63** in one step and high yield, making this a highly competitive alternative to the existing synthesis of the *N*-acyl equivalent **3.1** shown in Scheme 3.21.



Scheme 3.21 *Competing literature protocols*

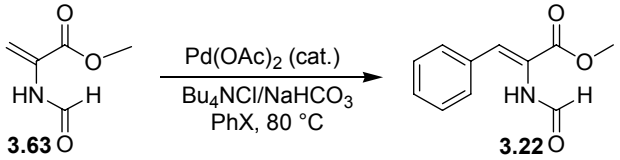
The protocol proposed and patented by Nugent provided a 1:1 mixture of mono- (**3.1**) and di-acetylated (**3.68**) products of which no yield was reported and that required separation.[58] In a later report, RajanBabu and coworkers proposed a

similar protocol and methyl 2-(acetamido)acrylate (**3.1**) was isolated from the mono- and di-acetylated mixture in 38% yield.[56d]

3.9.2 Use of 2-(formamido)acrylic methyl ester (3.63) in Heck reactions

Having achieved an efficient protocol for the preparation of **3.63**, methyl (*Z*)-2-(formamido)cinnamate (**3.22**) was prepared reacting **3.63** under Heck reaction conditions (Table 3.3). First the literature conditions, used by RajanBabu et al. with methyl 2-(acetamido)acrylate (**3.1**), were applied to **3.63** (11.5% palladium acetate).[56d]

Table 3.3 *Reaction of methyl 2-(formamido)acrylate (**3.63**) under Heck reaction conditions*



entry	Pd (%)[a]	PhX	*Z*:*E*[b]	yield (%)[c]
1	11.5	PhBr	28:1	59
2	11.5	PhI	12:1	68
3	5.0	PhBr	12:1	64
4	5.0	PhI	11:1	73
5	2.0	PhBr	13:1	45
6	2.0	PhI	13:1	73

[a]Reactions performed in a sealed tube at 80 °C for 22 h, [b]*Z*:*E* ratio determined by ^{1}H NMR. [c]Isolated yields, not optimized.

Product **3.22** was obtained in higher yield when using phenyl iodide (Table 2, entry 2), than when the less reactive phenyl bromide was used (entry 1). The amount of palladium acetate was subsequently lowered to 5% and 2%. Interestingly, yields slightly increased when using phenyl iodide (entries 4 and 6 vs. entry 2). Reducing the amount of $Pd(OAc)_2$ in the case of the less reactive phenyl bromide, eventually slowed down the reaction so much (entry 5) that polymerization processes started to be predominant as material with rubber consistency was found in the crude reaction mixture. This phenomenon was not observed in the other reactions. Good regioselectivities were obtained, the *Z* isomer being the major product in all cases.

3.10 Asymmetric hydrogenation of *N*-formyl α-dehydroamino esters

The *N*-formyl α-dehydroamino esters prepared (Figure 3.13) were used in the asymmetric Rh-catalyzed hydrogenation reaction employing chiral phosphoramidites as ligands (Figure 3.14).

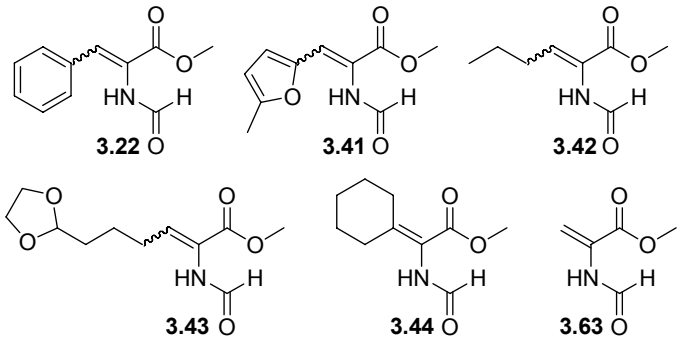


Figure 3.13 *Substrates used in this study*

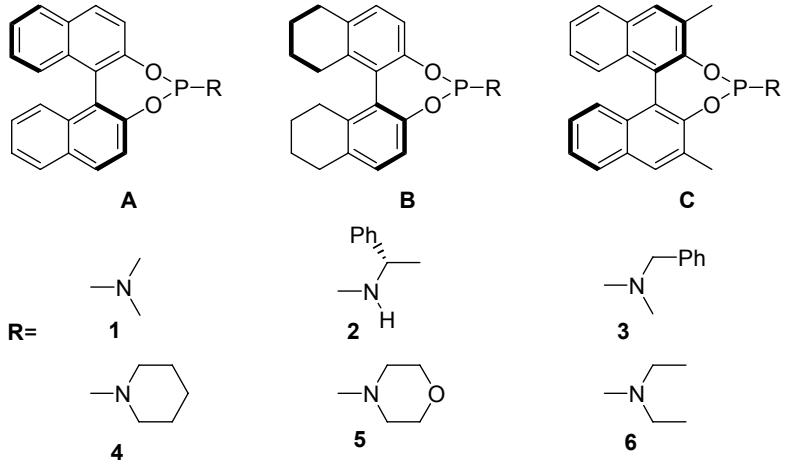
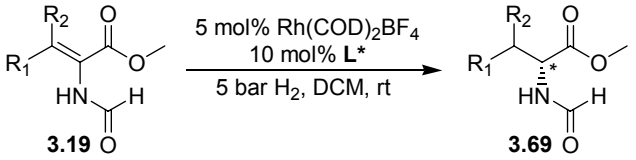


Figure 3.14 *Phosphoramidite ligands used in this study*

For screening, a catalyst loading of 5% was employed. Dichloromethane was used as solvent, as it previously had proven to be the solvent of choice.[12b] Among the ligand tested, PipPhos (**A4**) was chosen as it showed superior performance in terms of reactivity and enantioselectivity with many other substrates.[12e,g] Both *Z* and *E* isomers of the substrates shown in Figure 3.13 were hydrogenated. All reactions went to full conversion and the results are listed in Table 3.4.

Table 3.4 *Asymmetric hydrogenation of N-formyl α-dehydroamino methyl esters*

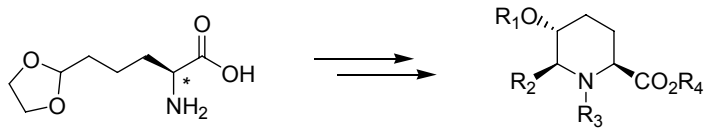


entry	substrate[a]	product	ligand	ee (%)[b,c]
1	**3.22 (*Z*)**	**3.23**	(*S*)-**A4**	**99** (*R*)
2	**3.22 (*E*)**	**3.23**	(*S*)-**A4**	46 (*R*)
3	**3.41 (*Z*)**	**3.70**	(*S*)-**A4**	**94** (*R*)
4	**3.41 (*Z*)**	**3.70**	(*S*)-**A1**	75 (*R*)
5	**3.41 (*Z*)**	**3.70**	(*S*)-**A5**	88 (*R*)
6	**3.41 (*E*)**	**3.70**	(*S*)-**A4**	64 (*R*)
7	**3.42 (*Z*)**	**3.71**	(*S*)-**A4**	**>99** (*R*)
8	**3.42 (*Z*)**	**3.71**	(*S*)-**A1**	98 (*R*)
9	**3.42 (*E*)**	**3.71**	(*S*)-**A4**	96 (*R*)
10	**3.43 (*Z*)**	**3.72**	(*S*)-**A4**	**>99** (*R*)
11	**3.43 (*E*)**	**3.72**	(*S*)-**A4**	97 (*R*)
12	**3.44**	**3.73**	(*S*)-**A4**	61 (*R*)
13	**3.63**	**3.74**	(*S*)-**A4**	**>99** (*R*)
14	**3.63**	**3.74**	(*S*)-**A1**	97 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate for 16 h. Conversions determined by ^{1}H NMR and GC. [b]ee's determined by chiral GC. [c]In all cases, the (S)-enantiomer of the ligand was used.

We were pleased to see that both *N*-formyl phenylalanine methyl ester (**3.23**) and *N*-formyl alanine methyl ester (**3.74**) were obtained with excellent enantioselectivity (>99% ee). This proved our hypothesis that the formyl group is comparable to the acyl group in directing the enantioselectivity, at least for this catalyst.[59] A high 94% ee (Table 3, entry 3) was obtained for the bulky heterocyclic substrate **3.41** (*Z*). Using **A1** (MonoPhos^{TM}) and **A5**, the same product **3.70** was obtained in only 75% and 88% ee, respectively (entries 4 and 5).[60] A class of substrates that has not been used before in Rh-catalyzed hydrogenation using monodentate ligands are β-alkyl substituted α-dehydroamino esters.[61] Excellent results (>99% ee in both cases) were obtained for substrates **3.42** (*Z*) and **3.43** (*Z*) using **A4** (entries 7 and 10).[62]

In contrast to the high enantioselectivity achieved for the *Z* isomers, using bidentate phosphine ligands the hydrogenation of the *E* isomers usually proceeds at much lower rates and gives poor enantioselectivities.[63,64] The results of the hydrogenation of the *E* isomer of aryl-substituted substrates **3.22** and **3.41** seem to confirm this trend also for monodentate phosphoramidites (entries 2 and 6). Products **3.23** and **3.70** were obtained with enantioselectivities of 46% and 64% respectively, without change in the configuration. Remarkably, the hydrogenation of the *E* isomer of alkyl-substituted substrates **3.42** and **3.43** proceeded with much higher enantioselectivities (96% and 97% ee, entries 9 and 11).[65] These results are particularly important as alkyl α-dehydroamino acid derivatives are difficult to prepare in geometrically pure form. The excellent results obtained for product **3.72** are very interesting as this compound bears an additional functionality, which could be used for further transformations, as demonstrated by the work presented by Blaauw and coworkers on the homologous **3.75** (Scheme 3.22).[66]



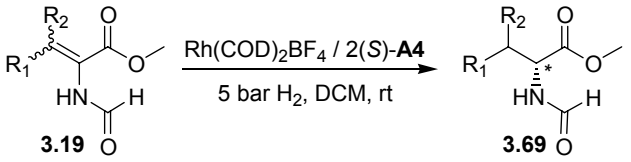
3.75 **3.76**

Scheme 3.22 *Transformation of a homologous of **3.72** into a tetrahydropyridine derivative **3.76***

Due to the excellent results obtained with substrates **3.22**, **3.41**-**3.43** and **3.63**, it was decided to test the activity of the system by lowering the catalyst loading using **A4** as ligand (Table 3.5). The catalyst loading could be decreased to 1 mol% for most *Z* isomers and methyl 2-(formamido)acrylate (**3.63**) (Table 3.5, entries 1, 3, 6, 8) without compromising reactivity and enantioselectivity. Under these conditions, the hydrogenation of methyl 2-(formamido)acrylate (**3.63**) and of the corresponding alkyl-substituted derivatives **3.42** (*Z*) and **3.43** (*Z*) were finished within 15 minutes, according to the hydrogen uptake.[67]

On the other hand, for substrates **3.42** (*E*) and **3.43** (*E*) the catalyst loading could be lowered to 2 mol% maintaining good enantioselectivities (entries 4 and 8). When **3.42** (*E*) was hydrogenated using 1 mol% of catalyst a decrease in enantioselectivity was observed together with an incomplete conversion (entry 4 vs. 5). This means that the use of 2 mol% of catalyst allows the hydrogenation of mixtures of *Z* and *E* isomers of alkyl-substituted α-dehydroamino esters affording full conversion and excellent ee's. The *E* isomers of substrates **3.22** and **3.41** have not been tested at lower catalyst loading due to the lower enantioselectivities previously obtained (Table 3.4, entries 2 and 6). No influence of the presence of *trans* and *cis* rotamers of the formamido group was noticed.

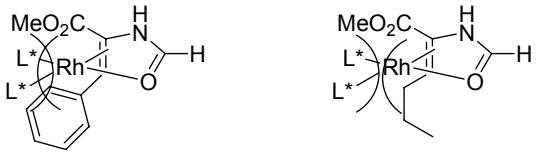
Table 3.5 *Asymmetric hydrogenation of N-formyl* α*-dehydroamino methyl esters: optimization of the catalyst loading*



entry	substrate	product	catalyst (mol%)[a]	conv. (%)	ee (%)[b,c]
1	**3.22 (*Z*)**	**3.23**	1	100	98 (*R*)
2	**3.41 (*Z*)**	**3.70**	2	>99	93 (*R*)
3	**3.42 (*Z*)**	**3.71**	1	100	>99 (*R*)
4	**3.42 (*E*)**	**3.71**	2	100	93 (*R*)
5	**3.42 (*E*)**	**3.71**	1	65	90 (*R*)
6	**3.43 (*Z*)**	**3.72**	1	100	>99 (*R*)
7	**3.43 (*E*)**	**3.72**	2	100	96 (*R*)
8	**3.63**	**3.74**	1	100	>99 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate for 16 h. Conversions determined by ^{1}H NMR and GC. [b]ee's determined by chiral GC. [c]In all cases, the (S)-enantiomer of the ligand was used.

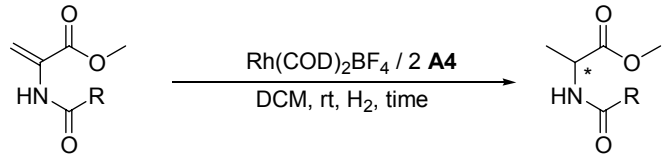
According to Scott and coworkers, the superior results obtained with *E* isomers of alkyl substrates compared to *E* isomer of aryl substrates suggest less interference in the coordination to the metal center as represented in Scheme 3.23.[63b] The flexibility of the alkyl chain might be the reason for the good reactivities and enantioselectivities still obtained.



Scheme 3.23 *Schematic representation of the different steric hindrance caused by the aryl or alkyl substituents present in the E isomers*

In order to test the efficiency of the hydrogenation of methyl 2-(formamido)acrylate (**3.63**) the catalyst loading was further decreased to 0.2 mol%, the hydrogen pressure varied from 5 to 1 bar and **A4** was used as chiral ligand. Identical reactions were performed using methyl 2-(acetamido)acrylate (**3.1**) as a comparison (Table 3.6).

Table 3.6 *Asymmetric hydrogenation of methyl 2-(formamido)acrylate (**3.63**) and methyl 2-(acetamido)acrylate (**3.1**) at lower catalyst loading and different pressure*



3.63: R= H
3.1: R= CH_3

3.74: >99% ee
3.2: >99% ee

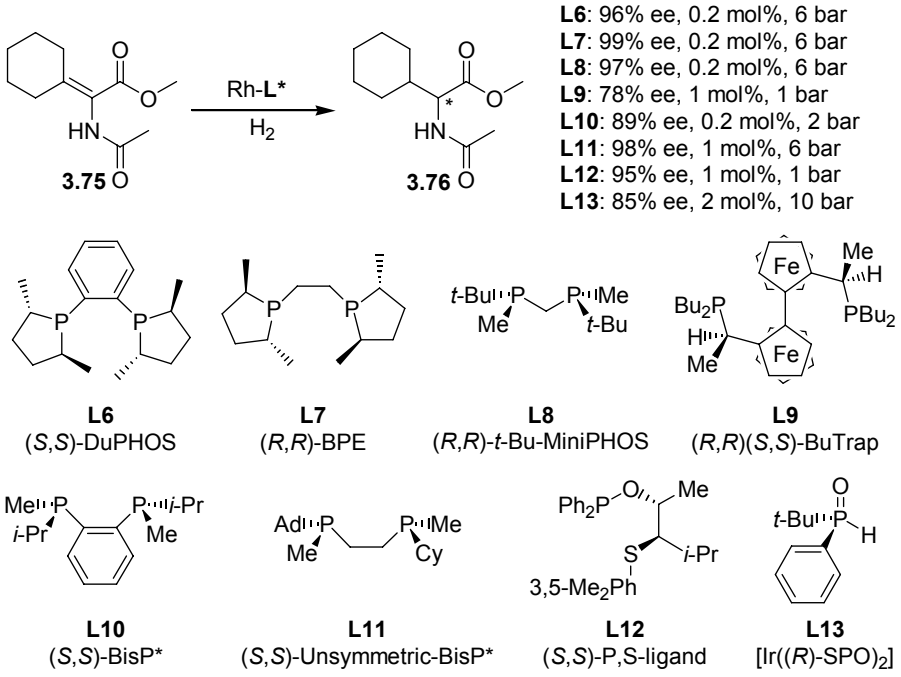
entry	compound	product	ligand[a,b]	pressure (bar)	time (min)[c,d,e]
1	**3.63**	**3.74**	(*S*)-**A4**	5	15
2	**3.63**	**3.74**	(*S*)-**A4**	2	55
3	**3.63**	**3.74**	(*S*)-**A4**	1	60 (64% conv.)
4	**3.1**	**3.2**	(*S*)-**A4**	5	15
5	**3.1**	**3.2**	(*S*)-**A4**	2	25
6	**3.1**	**3.2**	(*S*)-**A4**	1	60

[a]Reactions performed in 4 mL of solvent with 1.0 mmol of substrate and 0.2 mol% of catalyst. [b]In all cases, the (*S*)-enantiomer of the ligand was used. [c]Conversion and ee's determined by chiral GC. [d]Reaction time determined according to the hydrogen uptake. [e]Full conversion was obtained in all cases, unless indicated.

In all cases, the enantioselectivity remained excellent (>99% ee) for both substrates. Full conversion was still achieved with 2 bar hydrogen pressure; even if the hydrogen uptake showed that the hydrogenation of **3.63** started to be slower than the one of **3.1**, but was completed within the hour (entries 2 and 5). When the pressure was further reduced to 1 bar, full conversion was still obtained in one hour for **3.1** whilst **3.63** reached only 64% conversion.

3.11 β,β-Disubstituted α-dehydroamino esters

β,β-Disubstituted α-dehydroamino acids derivatives are known to be problematic substrates and the few ligands reported to induce good enantioselectivities in their hydrogenation are depicted in Scheme 3.24.[11b]

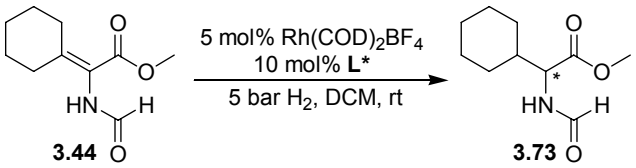


Scheme 3.24 *Enantioselective hydrogenation of methyl 2-(acetamido)-3,3-cyclohexylidene acetate (**3.75**)*

The first example of selective and efficient hydrogenation of the *N*-acyl equivalent **3.75** was reported by Burk and coworkers using DuPHOS (**L6**) and BPE-type ligands (**L7**). Enantioselectivities of 96% and 99%, respectively, were obtained.[68a] Other examples reported include bidentate diphosphines ligands such as *t*-Bu-MiniPhos (**L8**, 97% ee),[68b] BuTrap (**L9**, 78% ee),[68c] symmetric BisP* (**L10**, 89% ee),[68d] unsymmetric BisP* (**L11**, 98% ee)[68e] and the P,S ligands of Campos and coworkers (**L12**, 95% ee).[68f] The sole report of a monodentate ligand describes the use of a chiral secondary phosphine oxide (**L13**, 85% ee) using $[Ir(COD)Cl]_2$ as metal precursor, although long reaction time was required to reach full conversion (69 hours).[69]

Indeed, the hydrogenation of methyl 2-(formamido)-3,3-cyclohexylidene acetate (**3.44**) turned out to be more challenging. Using PipPhos (**A4**) as ligand, the reaction went to completion but afforded **3.73** with only 61% ee (Table 3.4, entry 12). Due to the moderate enantioselectivity obtained it was decided to test a few more phosphoramidites ligands maintaining the same conditions. The results are listed in Table 3.7.

Table 3.7 *Asymmetric hydrogenation of methyl 2-(formamido)-3,3-cyclohexylidene acetate (**3.44**)*

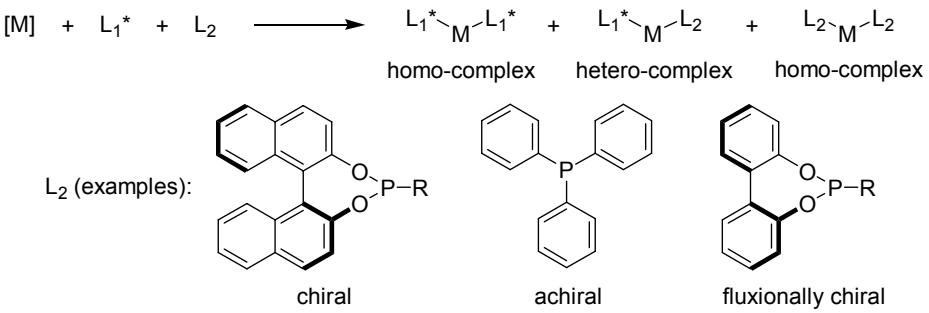


entry	ligand	conv. (%)[a]	ee (%)[b,c]
1	(*S*)-**A4**	100	61 (*R*)
2	(*S*)-**A1**	93	25 (*R*)
3	(*S*)-**A5**	54	42 (*R*)
4	(*S*)-**B4**	100	41 (*R*)
5	(*R*)-**C4**	35	62[d] (*S*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate for 16 h. Conversions determined by 1H NMR and GC. [b]ee's determined by chiral GC. [c]In all cases, the (*S*)-enantiomer of the ligand was used. [d]In this case, the (*R*)-enantiomer of the ligand was used.

It turned out that PipPhos (**A4**) was the best ligand both in terms of reactivity and enantioselectivity (Table 3.7, entry 1), among the ligands tested. Full conversion was achieved also using **B4** but with considerable loss in enantioselectivity (entry 4). Comparable enantioselectivity (62% ee) was obtained using **C4**, but the ligand was clearly too bulky as only 35% conversion was reached (entry 5). The use of **A1** as chiral ligand afforded the lowest enantioselectivity (entry 2). Previously in this research group, the use of **A1** under the same reaction conditions afforded the acetamido equivalent **3.76** with a similar conversion (95% conv.) but even lower enantioselectivity (17% ee).[70]

In order to improve the results obtained in the hydrogenation of **3.44**, combinations of ligands were applied (Scheme 3.25).[71] The assumption was that the combination of two different ligands might confer to the complex a chiral environment suitable enough to force the substrate in a more favorable coordination to the metal center and that this might induce better results both in terms of reactivity and enantioselectivity compared to their homo-combination. Moreover, for the ligand combination to be effective, the hetero-complex should be preferentially formed or be considerably more reactive and selective than each of the homo-complexes. The possibility of the mixing of different ligands can be considered another interesting advantage arising from the use of monodentate ligands. This broadens the ligand differentiation and gives the opportunity to easily access Rh-complexes that are not symmetric.



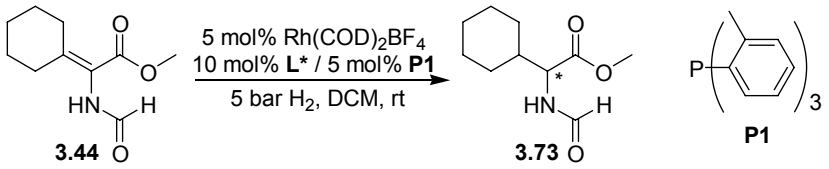
Scheme 3.25 *Hetero- and homo-combination of monodentate ligands*

The potential of mixing chiral monodentate ligands with other chiral,[72] achiral[73a,12f] or fluxionally chiral[73b,c] monodentate ligands has been recently elucidated by the groups of Reetz, Piarulli/Gennari and by our group.[74] The method has been applied not only in Rh-catalyzed hydrogenation and hydroformylation reactions,[72-73,12f] but also in Rh-catalyzed boronic acid addition.[75] Nevertheless, even if an influence of this approach on the results has been often noticed, improvements on the results obtained using a homo-complex have not always been reported.

Therefore, due to the recent successful application in our laboratories of this concept in the hydrogenation of cinnamic acids,[12f] it was decided to employ a combination of chiral phosphoramidites and achiral phosphines (with a ratio of 2:1). The phosphoramidites used were also tested without addition of phosphine in order to be able to appreciate their influence. The results of this investigation are shown in Table 3.8 and Table 3.9.

First, PipPhos (**A4**) was tried in combination with tri-*ortho*-tolylphosphine (**P1**) (Table 3.8, entry 2). Pleasingly, an increase in enantioselectivity was observed compared to the homo-combination (entry 1), although the reaction was slightly slower. The same increase in enantioselectivity but decrease in activity was observed for MonoPhos™ (**A1**) (entries 5 and 6). On the contrary, when the 3,3'-dimethyl-BINOL version of PipPhos (**C4**) was used, an increase both in terms of reactivity and enantioselectivity was observed (entries 3 vs 4). Very excitingly, the hetero-combination of **C4** and tri-*ortho*-tolylphosphine (**P1**) provided full conversion to the product and a remarkable 85% ee, as the best result so far. The influence of the phosphine resulted to be less dramatic in the case of the 3,3'-dimethyl-BINOL version of MonoPhos™ (**C1**) (entries 7 vs 8), where only a slight increase in enantioselectivity was achieved. When another 3,3'-dimethyl substituted phosphoramidite ligand was used (**C6**), a somewhat higher conversion to the product was observed for the hetero-combination, but with the same degree of enantioselectivity (entries 9 and 10). It should be noticed that the homo-combinations of **C1** and **C6** showed better enantioselectivity than PipPhos (**A4**) although the catalyst is slower (entries 7 and 9 vs 1).

Table 3.8 *Asymmetric hydrogenation of methyl 2-(formamido)-3,3-cyclohexylidene acetate (**3.44**) with hetero-combination of phosphoramidites and tri-ortho-tolylphosphine (**P1**) as ligands.*



entry	ligand	phosphine	conv. (%)[a]	ee (%)[b]
1	(*S*)-**A4**	-	100	61 (*R*)
2	(*S*)-**A4**	**P1**	95	70 (*R*)
3	(*R*)-**C4**	-	35	62 (*S*)
4	(*R*)-**C4**	**P1**	**100**	**85** (*S*)
5	(*S*)-**A1**	-	93	25 (*R*)
6	(*S*)-**A1**	**P1**	40	48 (*R*)
7	(*S*)-**C1**	-	97	78 (*R*)
8	(*S*)-**C1**	**P1**	**98**	**82** (*R*)
9	(*R*)-**C6**	-	76	74 (*S*)
10	(*R*)-**C6**	**P1**	87	74 (*S*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate, 0.01 mmol of $Rh(COD)_2BF_4$, 0.02 mmol of chiral phosphoramidite and 0.01 mmol of achiral phosphine (if present). Reactions were run for 16 h. [b]ee's determined by chiral GC.

In consideration of the good results obtained with **C4** and tri-*ortho*-tolylphosphine as ligands, it was decided to test additional phosphines (Figure 3.15). The outcome of this further investigation is shown in Table 3.9.

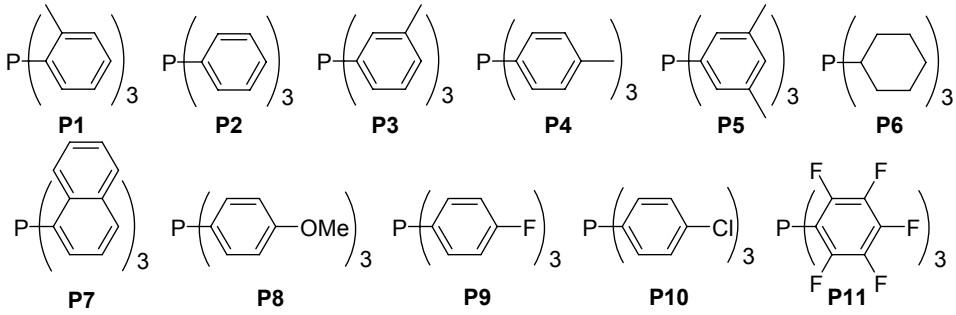
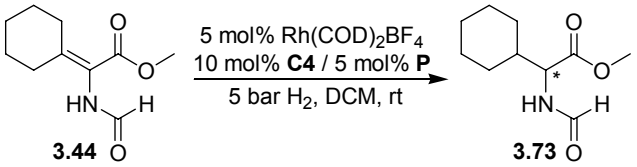


Figure 3.15 *Phosphine ligands used in the hetero-complexes*

Table 3.9 *Asymmetric hydrogenation of methyl 2-(formamido)-3,3-cyclohexylidene acetate (**3.44**) with hetero-combination of ligand **C4** and different phosphines (**P**).*



entry	ligand	phosphine	conv. (%)[a]	ee (%)[b,c]
1	(*R*)-**C4**	-	35	62 (*S*)
3	(*R*)-**C4**	**P1**	**100**	**85** (*S*)
3	(*R*)-**C4**	**P2**	100	69 (*S*)
4	(*R*)-**C4**	**P3**	100	72 (*S*)
5	(*R*)-**C4**	**P4**	100	59 (*S*)
6	(*R*)-**C4**	**P5**	100	68 (*S*)
7	(*R*)-**C4**	**P6**	93	36 (*R*)
8	(*R*)-**C4**	**P7**	100	71 (*S*)
9	(*R*)-**C4**	**P8**	100	62 (*S*)
10	(*R*)-**C4**	**P9**	100	72 (*S*)
11	(*R*)-**C4**	**P10**	100	65 (*S*)
12	(*R*)-**C4**	**P11**	57	73 (*S*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate, 0.01 mmol of $Rh(COD)_2BF_4$, 0.02 mmol of chiral phosphoramidite and 0.01 of mmol achiral phosphine. Reactions were run for 16 h. [b]ee's determined by chiral GC.

Complete conversion was achieved in almost all cases. Interestingly, using tri-cyclohexylphosphine (**P6**) the opposite configuration of the product was obtained. The influence of the presence and position of the methyl substituent of the achiral phosphine on the enantioselectivity was tested by comparing triphenylphosphine (**P2**), tri-*ortho*, *meta*, *para*-tolylphosphine (**P1**, **P3**, **P4**) and tri-3,5-dimethylphenylphosphine (**P5**) (Table 3.9, entries 2-6). The enantioselectivity obtained with **P1** remained the highest. Lower and comparable results were achieved with or without methyl substituent in the other positions. Only by using tri-*para*-tolylphosphine (**P4**) a consistent decrease was observed. Moreover, the hydrogen uptake showed an opposite trend between reactivity and enantioselectivity, with tri-*ortho*-tolylphosphine (**P1**) being the slowest (85% ee, 16 h) and tri-*para*-tolylphosphine the fastest (**P4**) (59% ee, around 4 h). Electron-donating or withdrawing substituents seemed to have little influence on the

enantioselectivity (entries 9-11) compared to tri-phenylphosphine (**P2**). In all three cases reaction rate appeared to be comparable to the one observed with **P4**, with the *para*-methoxy **P8** slightly faster than, respectively, *para*-fluoro **P9** and *para*-chloro **P10**. As a conclusion, the results suggest that the sterics of the phosphine substituents play a larger role than their electronic properties.

Due to the good result obtained when using **C4** and **P1**, an attempt was made to reduce the catalyst loading in the hydrogenation of **3.44**. Nevertheless, the use of 2 mol% catalyst under 25 bar hydrogen pressure led to an incomplete conversion (64%) and lower enantioselectivity (81% ee). Therefore, at the moment the best conditions seem to be still the use of 5 mol% of catalyst.

An induction period of more than one hour was observed for the hydrogenation reaction catalyzed using **C4** and **P1** as ligands. The reason for this delay could not be clarified, but the reverse proportionality between reactivity and enantioselectivity, showed by the reactions in which the three different tri-tolylphosphines **P1**, **P3** and **P4** were used, might suggest an involvement of the increasing steric hindrance of the complexes (Figure 3.16).

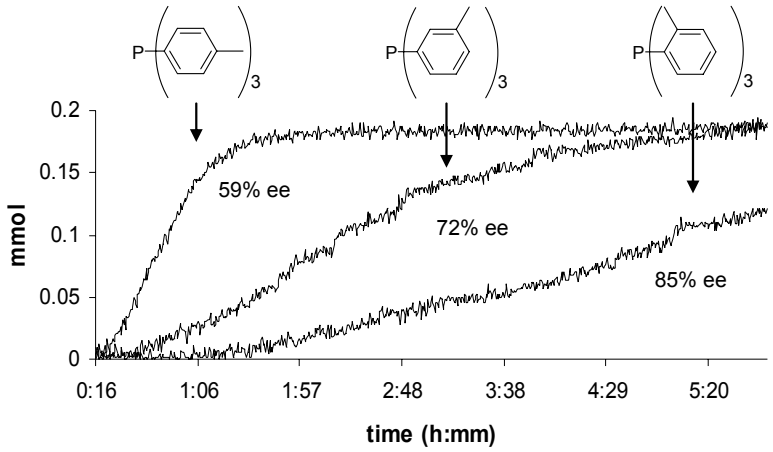


Figure 3.16 *Hydrogen uptake profiles for the asymmetric hydrogenation reactions of **3.45** using **C4** and **P1**, **P3**, **P4** as ligands (Table 3.9, entries 3-5)*

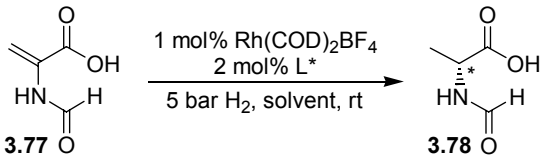
Not knowing the actual structure of these hetero-complexes increases the difficulties of making any kind of assumption without further investigations. What seems to be clear is that the hetero-complex suits the characteristics of this bulky substrate. A bulkier catalyst might lead to a more defined and therefore selective but less accessible complex for the already bulky substrate **3.7**. It is also difficult to predict at which stage of the reaction this has the highest influence, as the Rh(I)-complex is square planar and the Rh(III)-complex obtained after oxidative addition of H_2 is octahedral.

The induction time might suggest that the slow process taking place is associated with the formation of the hetero-complex. Luckily the homo-complex comprising two molecules of **C4** as ligand is a very slow catalyst (35% conv., Table 3.9, entry 1), it would be interesting to see whether reactivity and enantioselectivity would improve given the possibility of using a preformed hetero-complex.

3.12 Hydrogenation of 2-formamidoacrylic acid (3.77)

In order to show that not only esters but also *N*-formyl α-dehydroamino acids can be successfully hydrogenated, hydrogenation of 2-formamidoacrylic acid (**3.77**)[76] was studied using MonoPhos™ (**A1**)[77] as chiral ligand (Table 3.10). Dichloromethane turned out to be the best solvent and an enantioselectivity of 92% was obtained. In the same solvent the corresponding methyl ester **3.63** was hydrogenated yielding 97% ee (Table 3.4, entry 15). Lower conversion and enantioselectivity were obtained with ethyl acetate (entry 2) which instead was the best solvent using 2-acetamidoacrylic acid (99% ee).[8d]

Table 3.10 *Asymmetric hydrogenation of 2-formamidoacrylic acid (**3.77**)*



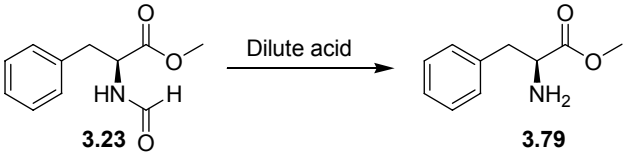
entry	ligand	solvent	conv. (%)[a,b]	ee (%)[c]
1	(*S*)-**A1**	DCM	100	92 (*R*)
2	(*S*)-**A1**	EtOAc	70	89 (*R*)
3	(*S*)-**A1**	*i*-PrOH	85	53 (*R*)
4	(*S*,*R*)-**A2**	DCM	21	54 (*R*)
5	(*S*)-**A3**	DCM	96	86 (*R*)

[a]Reactions performed in 4 mL of solvent with 0.2 mmol of substrate overnight. [b]Conversions determined by ^{1}H NMR and GC. [c]ee's determined by chiral GC after conversion to methyl ester **3.74**.

3.13 *N*-formyl: from directing to protecting group

In order to establish the utility of the formyl moiety as protecting group, it is necessary to prove its superiority compared to the acyl functionality during the deprotection step. As final part of our investigation, the removal of the formyl protection was studied. *N*-formyl phenylalanine methyl ester (**3.23**)[78] was converted into the corresponding amine under mildly acidic conditions (Table 3.11).

Table 3.11 *Removal of N-formyl protection under mildly acidic conditions*



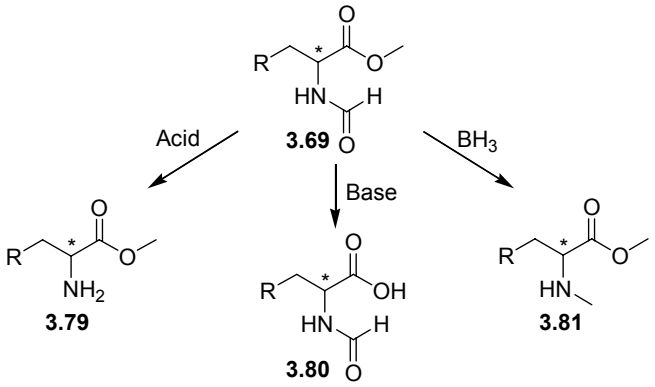
3.23 **3.79**

entry	conditions	yield (%)	ee (%)[a]
1	HCl (1 eq), MeOH reflux, 1h	>99	>99
2	HCl (1 eq), MeOH rt, 8h	>99	>99
3	H_3PO_4 (1.5 eq), THF, reflux, 9h	89	>99

[a]ee's determined by chiral GC after *N*-formyl protection of the product.

Interestingly, the formyl group is removed at room temperature in the presence of one equivalent of hydrochloric acid in methanol (Table 9, entry 2). These conditions are even milder than those previously reported by Sheehan and Yang.[32a] No reaction was observed applying the same conditions to the *N*-acyl protected α-amino ester **3.4**. Alternatively, the formyl group was removed using recently reported conditions for Boc deprotection, with the weaker acid H_3PO_4 (entry 3).[79] In all cases no racemization was observed as it was established after *N*-formyl protection of **3.79**. Moreover, the formyl group is remarkably resistant toward basic hydrolysis and azlactone formation.[80] The orthogonal removal of formyl and ester group makes a stepwise approach toward the synthesis of peptides possible (Scheme 3.26).

Another interesting feature connected with the use of *N*-formyl α-amino esters **3.69** is their convenient transformation, in one step via borane reduction, into *N*-methyl α-amino acid derivatives **3.81** (Scheme 3.26).[81] The properties exhibited by various natural products in which *N*-methyl α-amino acids are present, makes them of great interest from a medicinal and synthetic point of view.[82]



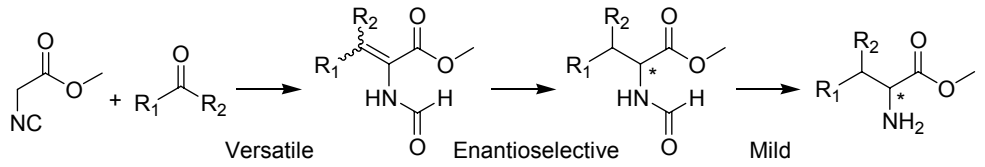
3.69 **3.79** **3.80** **3.81**

Scheme 3.26 *Transformations of N-formyl* α*-amino esters (**3.69**)*

3.14 Conclusions

We have demonstrated that *N*-formyl protected α-dehydroamino esters **3.19** are excellent substrates for Rh-catalyzed asymmetric hydrogenation in combination with monodentate phosphoramidites as ligands. Excellent enantioselectivities (up to >99% ee) were obtained for the *Z* isomers. An important finding is that high enantioselectivities can also be achieved (up to 97% ee) for the *E* isomers of substrates with alkyl substituents (**3.42**, **3.43**). Good results were obtained for a β,β-disubstituted substrate (**3.44**, up to 85% ee) by using combinations of phosphoramidites and achiral phosphines.

The use of *N*-formyl protection allows an efficient one-pot synthesis of a large variety of substrates, both in terms of possible functionalities tolerated and ease of preparation. Finally, the protecting group can be easily removed under mild reaction conditions (Scheme 3.27).



Scheme 3.27 *An efficient approach to a variety of* α*-amino acid derivatives*

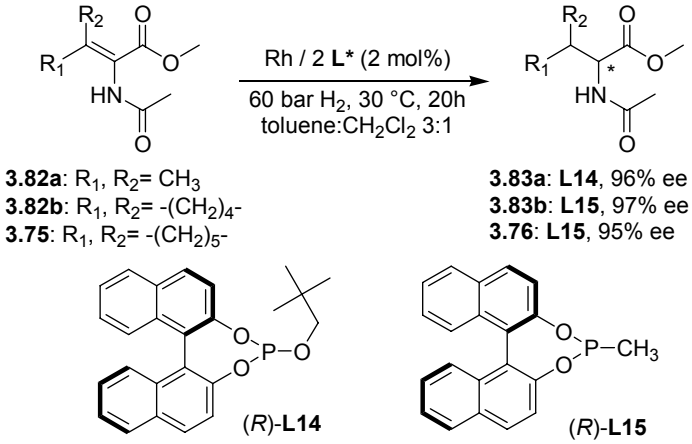
An inexpensive, efficient and multigram scale protocol for the synthesis of methyl 2-(formamido)acrylate (**3.63**) was developed. This makes the Heck reaction even more interesting as methodology for the preparation of aromatic α-dehydroamino esters **3.7**. This procedure has the advantage of affording preferentially the *Z* isomers of the desired products, which becomes important for aromatic α-dehydroamino esters as the *E* isomers cannot be efficiently hydrogenated (Table 3.4, **3.22** and **3.41**).

Based on these results, it is evident that the *N*-formyl protection is a very versatile, useful and underestimated synthetic tool and provides a link between optically active α-amino acids preparation and asymmetric hydrogenation.

3.15 Outlook

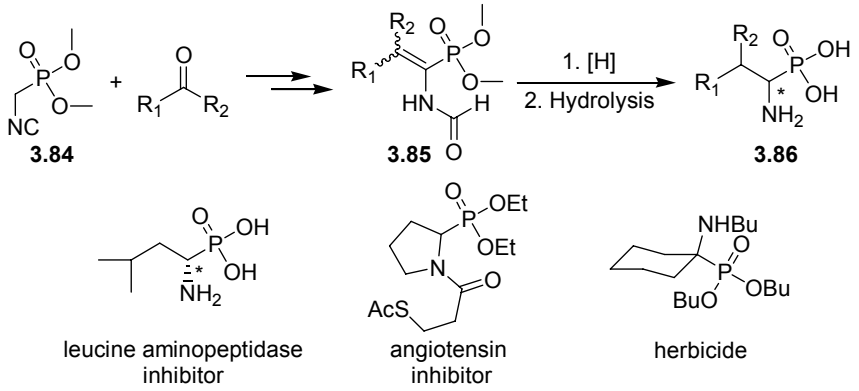
During the preparation of this manuscript, Reetz reported enantioselectivities up to 97% ee in the asymmetric hydrogenation of β,β-disubstituted methyl 2-(acetamido)acrylates **3.82a-b** and **3.75** using monodentate phosphorus based ligands. The best results obtained using monodentate phosphite **L14** and phosphinite ligand **L15** are depicted in Scheme 3.28.[83] Interestingly, when **3.82a** was hydrogenated in the presence of phosphoramidites **A1**, **A2** and **A4**,

incomplete conversions (4-85% conv.) and low enantioselectivities were obtained (4-46% ee).[84]



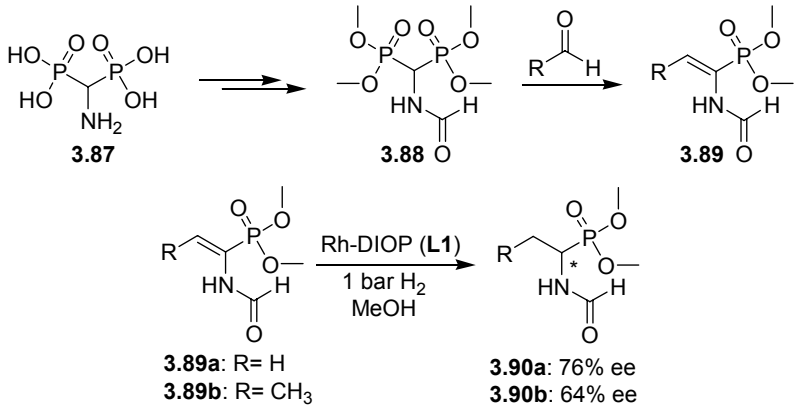
Scheme 3.28 *Asymmetric hydrogenation of β,β-disubstituted methyl 2-(acetamido)acrylates **3.82a-b** and **3.75** using monodentate ligands*

Looking for new and interesting substrates for Rh-catalyzed asymmetric hydrogenation reactions, an aspect worth considering is that the Schöllkopf synthesis of *N*-formyl α-dehydroamino phosphonates[85] **3.85** via 2-oxazolines provides an interesting synthetic pathway for the preparation of enantiopure α-amino phosphoric acids **3.86**. These compounds and their derivatives, for which only few efficient synthetic procedures are available,[86] are known to be important α-amino acid mimetics (Scheme 3.29).[87]



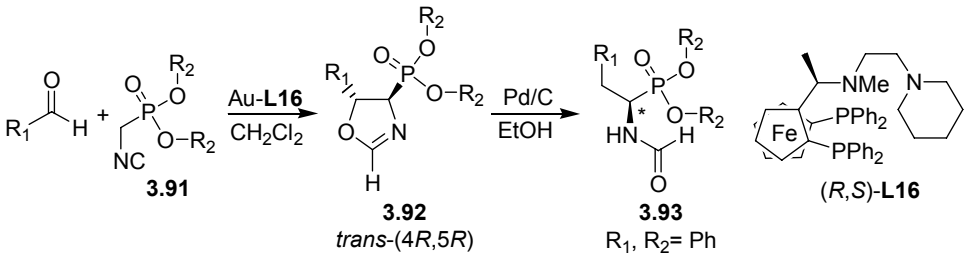
Scheme 3.29 *Proposed route to bioactive α-amino phosphoric acid derivatives **3.86** via Schöllkopf 2-oxazolines and asymmetric hydrogenation*

Schöllkopf and coworkers reported an interesting example for the preparation and hydrogenation of *N*-[1-(dimethoxyphosphoryl)ethenyl]formamide (**3.89a**) and *N*-[1-(dimethoxyphosphoryl)propenyl]formamide (**3.89b**). Enantioselectivities of 76% and 64% were obtained using DIOP (**L1**) as chiral ligand (Scheme 3.30).[88]



Scheme 3.30 *Asymmetric hydrogenation leading to α-amino phosphonates **3.90a-b** using DIOP (**L1**)*

Ito and coworkers also presented an asymmetric version of the condensation leading to optically active *trans*-5-alkyl-2-oxazoline-4-phosphonates (**3.92**) with enantioselectivities up to 96% using the Au(I)-ferrocenylphosphine (**L16**).[89] In a specific example, hydrogenolysis using Pd/C (50 bar H_2) was performed to convert **3.92** into its chiral α-amino phosphonate equivalent **3.93** (Scheme 3.31).



Scheme 3.31 *Asymmetric synthesis of trans-5-alkyl-2-oxazoline-4-phosphonates (**3.92**) using Au-**L16** complexes*

3.16 Experimental section

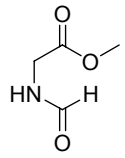
General Procedures.

For general remarks, see Chapter 2. Enantiomeric excesses and conversions were determined by capillary GC analysis on a HP 6890 gas chromatograph equipped with a flame ionization detector. All the monodentate phosphoramidite ligands have been previously described.[90,91]

The preparation of compounds **3.43** and **3.46** was performed by Alicia Marco Aleixandre. The preparation and hydrogenation of 2-(formamido)acrylic acid (**3.77**) and the temperature dependent ^{1}H-NMR study of methyl 2-(formamido)cinnamate (**3.22**) were performed by Gerlof J. Kruidhof. The preparation and deprotection of (*L*)-*N*-formyl phenyl alanine methyl ester (**3.23**) were performed by Jort Robertus.

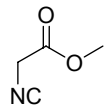
Starting Material Synthesis

***N*-Formyl glycine methyl ester (3.33)**[39]



To a solution of methyl formate (120 mL) containing glycine methyl ester hydrochloric salt (20.0 g, 0.159 mol) NEt_3 (17.7 g, 0.175 mol, 24.3 mL) was added. The reaction mixture was stirred for three days at room temperature, after which time the salts were filtered and the solution reduced to half the volume. After cooling the solution at -5 °C more salts were filtered and the solvent completely removed. Distillation of the crude mixture (105 °C, 0.15 Torr) afforded the desired product as a colorless oil (17.30 g, 93% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H, *trans*), 7.97 (d, *J*= 12.0 Hz, 1H, cis), 6.92 (br s, 1H, *trans*), 6.65 (br s, 1H, cis), 4.01 (s, 1H, *trans*), 3.99 (s, 1H, *trans*), 3.96 (s, 1H, cis), 3.94 (s, 1H, cis), 3.70 (s, 3H, cis), 3.69 (s, 3H, *trans*). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 169.9 (s), 165.0 (s), 52.2 (q), 39.6 (t).

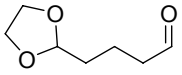
Methyl isocyanoacetate (3.20)[39]



To a solution of **3.33** (10.0 g, 85.4 mmol) and triethylamine (29.7 mL, 21.3 mmol, 2.5 eq.) in CH_2Cl_2 (200 mL) in a 500 mL flask, $POCl_3$ (8.0 mL, 85.4 mmol, 1 eq.) was added dropwise at 0 °C. The solution turned immediately red. After all the $POCl_3$ was added, the reaction mixture was stirred for an additional hour at room temperature before an aqueous solution of Na_2CO_3 (10.0 g, 200 mL of H_2O) was very carefully added to the reaction mixture, due to the conspicuous evolution of gas. The reaction mixture was stirred for 30 min. or untill the evolution of gas ceased. The organic phase was separated from the aqueous phase, washed with brine, and dried over K_2CO_3. Distillation of the crude mixture (75 °C, 10 Torr) afforded the desired product as a colorless liquid (7.50 g, 89% yield). R_f 0.61 (heptanes-ethyl acetate 1:1).

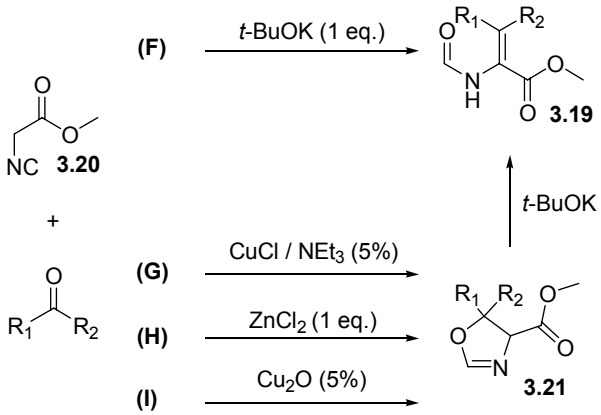
^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.23 (s, 3H), 3.81 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.3 (s), 161.1 (d), 53.2 (q), 43.2 (t).

5-(1,3-Dioxolan-2-yl)-1-pentanal (3.46)[92]



To a solution of 2,2'-trimethylene-bis-1,3-dioxolane (1.8 mL, 11.3 mmol) in acetone (54 mL) *p*-toluenesulfonic acid (0.38 g, 2.0 mmol) was added and the reaction mixture was stirred for 30 min. After that time, water (18 mL) was added and the mixture stirred for an additional 30 min. After the addition of a saturated aq. solution of $NaHCO_3$, the mixture was extracted with ether, the combined organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. Distillation of the crude of the reaction under reduced pressure (92 °C, 0.9 Torr) afforded the desired product as a colorless liquid (0.87 g, 46% yield), which was stored in the fridge (4 °C) protected by a layer of aluminum foil.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H), 4.84 (t, *J*= 4.5 Hz, 1H), 3.94-3.82 (m, 4H), 2.49 (t, *J*= 6.9 Hz, 2H), 1.72-1.67 (m, 4H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 202.0 (s), 104.2 (d), 103.9 (d), 64.7 (t), 43.4 (t), 33.5 (t), 32.8 (t), 18.4 (t), 16.3 (t).

Synthetic scheme for the preparation of *N*-formyl α-dehydroamino acid methyl esters (3.19)



Method F.[42a]

Methyl isocyanoacetate **3.20** (1 mL, 11.0 mmol) was added dropwise to a solution of freshly sublimed potassium *t*-butoxide (1.28 g, 11.0 mmol) in dry THF (8.3 mL) at -60 °C. The mixture was stirred for 15 min, after which time a solution of the aldehyde (11.0 mmol) in THF (6.9 mL) was added dropwise and stirring at -60 °C was continued for an additional 30 min. The reaction mixture was allowed to warm to 0 °C and after further 30 min stirring at 0 °C, a solution of acetic acid (0.65 mL, 11.0 mmol) in CH_2Cl_2 (27.5 mL) was added. The resulting suspension was extracted with water, the organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude mixture was purified by flash column

chromatography on silica gel (mixtures of heptanes-ethyl acetate) yielding the isolated *Z* and *E* isomers.

Method G.[93]

Triethylamine (77 µL, 0.55 mmol, 5 mol%) was added dropwise to a mixture of methyl isocyanoacetate **3.20** (1 mL, 11.0 mmol), aldehyde (13.2 mmol, 1.2 eq.) and CuCl (55 mg, 0.55 mmol, 5 mol%) in dry THF (8.5 mL) stirred at 0 °C. The reaction mixture was allowed to reach room temperature and was stirred overnight.[94] The reaction mixture was cooled to 0 °C and a solution of potassium *t*-butoxide (1.28 g, 11.0 mmol) in THF (10 mL) was added. After stirring for 30 min, acetic acid (0.65 mL, 11.0 mmol) in CH_2Cl_2 (27 mL) was added, the reaction mixture was allowed to reach room temperature and was extracted with water. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (mixtures of heptanes-ethyl acetate) yielding the isolated *Z* and *E* isomers.

Method H.[93]

A mixture of methyl isocyanoacetate **3.20** (1 mL, 11.0 mmol), cyclohexanone (1.37 mL, 13.2 mmol, 1.2 eq.) and $ZnCl_2$ (1.50 g, 11.0 mmol, 1 eq.) in dry THF (8.5 mL) was stirred overnight at room temperature. The reaction mixture was cooled to 0 °C and a solution of potassium *t*-butoxide (1.28, 11.0 mmol) in THF (10 mL) was added. After stirring for 30 min, acetic acid (0.65 mL, 11.0 mmol) in CH_2Cl_2 (27 mL) was added, the reaction mixture was allowed to reach room temperature and was extracted with water. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (heptanes-ethyl acetate 2:1) affording the desired product **3.7** (745 mg, 35% yield).

Method I.[95]

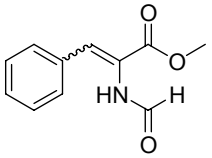
To a solution containing methyl isocyanoacetate **3.20** (1 mL, 11.0 mmol), aldehyde or ketone (13.2 mmol, 1.2 eq.) in dry ether (10 mL) Cu_2O (79 mg, 0.55 mmol, 5 mol%) was added in one portion causing an exothermic reaction. The mixture was stirred for 3 hours at room temperature, until TLC (heptanes-ethyl acetate 1:1) showed complete conversion of the starting material. At that point the temperature was lowered to 0 °C and a solution of potassium *t*-butoxide (1.28 g, 11.0 mmol) in THF (10 mL) was added. After stirring for 30 min, acetic acid (0.65 mL, 11.0 mmol) in CH_2Cl_2 (27 mL) was added, the reaction mixture was allowed to reach room temperature and was extracted with water. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (mixtures of heptanes-ethyl acetate).

Synthesis of methyl 2-(formamido)cinnamate (3.22) under Heck reaction conditions[96]

A mixture of iodobenzene (365 mg, 1.79 mmol), methyl 2-(formamido)acrylate (**3.63**) (305 mg, 2.13 mmol, 1.2 eq), $Pd(OAc)_2$ (8.0 mg, 0.036 mmol, 2 mol%), tetra-*N*-butyl ammonium chloride (599 mg, 2.16 mmol, 1.2 eq) and $NaHCO_3$ (407 mg, 4.84 mmol, 2.7 eq), was flushed with nitrogen and heated in a sealed tube at 80 °C for 22 h. Subsequently, the reaction mixture was cooled to room temperature and a mixture of CH_2Cl_2/H_2O (1:1, 100 mL) was added. The organic layer was washed with H_2O (25 mL) and the combined aqueous layers were extracted with CH_2Cl_2 (25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (heptanes-ethyl acetate 2:1) afforded the desired product **3.22** (*Z*/*E* 13:1, 268 mg, 73% yield).

Spectroscopic data

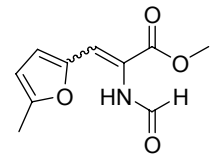
Methyl 2-(formamido)cinnamate (3.22)[97]



E isomer: Colorless oil, R_f 0.50 (heptanes-ethyl acetate 1:1), *trans*:cis = 75:25.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J*= 14.4 Hz, 1H, *trans*), 8.41 (d, *J*= 2.0 Hz, 1H, *trans*), 8.23 (s, 1H, *trans*), 7.61 (br, 1H, *trans*), 7.52 (br, 1H, cis), 7.42-7.22 (m, 5H), 6.92 (s, 1H, cis), 3.70 (s, 3H, cis), 3.65 (s, 3H, *trans*). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.7 (s), 159.2 (d), 135.1 (s), 129.3 (d), 128.7 (s), 128.8 (d, 2C), 128.1 (d), 127.8 (d, 2C), 52.4 (q). MS, *m/z (%)*: 205 (M^+, 28.6%); HRMS (EI^+) for $C_{11}H_{11}NO_3$, calcd: 205.074, found: 205.073.

Z isomer: White solid, R_f 0.46 (heptanes-ethyl acetate 1:1), *trans*:*cis* = 47:53. M.p. 92.4-93.0 °C (Lit. 88-89 °C).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H, *trans*), 8.06 (d, *J*= 11.2 Hz, 1H, *cis*), 7.92 (br, 1H, *trans*), 7.58 (d, *J*= 8.0 Hz, 1H, *cis*), 7.50-7.18 (m, 6H), 3.77 (s, 3H, *cis*), 3.71 (s, 3H, *trans*). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.3 (s, *trans*), 164.9 (s, *cis*), 163.8 (d, *cis*), 159.3 (d, *trans*), 133.4 (s, *trans*), 132.9 (d, *trans*), 132.6 (s, *cis*), 129.8 (d, *cis*), 129.6 (d), 129.5 (d), 129.1 (d), 129.0 (d, *trans*), 128.5 (d, *cis*), 123.9 (s, *cis*), 122.3 (s, *trans*), 52.9 (q, *cis*), 52.7 (q, *trans*). MS, *m/z (%)*: 205 (M^+, 31.0%); HRMS (EI^+) for $C_{11}H_{11}NO_3$, calcd: 205.074, found: 205.074.

Methyl 2-(formamido)-3-(5-methyl-furan-2-yl)acrylate (3.41)

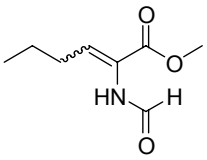


E isomer: Light yellow solid, R_f 0.38 (heptanes-ethyl acetate 1:1), *trans*:*cis* = 71:29. M.p.= 59.5-60.7 °C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J*= 2.0 Hz, 1H, *cis*), 8.33 (s, 1H, *trans*), 8.11 (s, 1H, *trans*), 7.55 (br, 1H, *trans*), 7.32 (br, 1H, *cis*), 7.30 (d, *J*= 3.2 Hz, 1H, *cis*), 7.07 (d, *J*= 2.8 Hz, 1H, *trans*), 6.70 (s, 1H, *cis*), 6.14 (d, *J*= 3.6 Hz, 1H, *cis*), 6.10

(d, *J*= 2.8 Hz, *trans*), 3.90 (s, 3H, *trans*), 3.87 (s, 3H, *cis*), 2.33 (s, 3H, *cis*), 2.32 (s, 3H, *trans*). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 163.6 (s), 162.0 (d, *cis*), 158.9 (d, *trans*), 154.4 (s), 147.54 (s), 118.8 (s), 118.1 (d, *cis*), 117.7 (d, *cis*), 117.5 (d, *trans*), 117.3 (d, *trans*), 109.4 (d, *cis*), 109.1 (d, *trans*), 52.3 (q), 13.8 (q). MS, *m/z (%)*: 209 (M^+, 59.9%); HRMS (EI^+) for $C_{10}H_{11}NO_4$, calcd: 209.069, found: 209.070. Elem. Anal.: calcd: C 57.41, H 5.30, N 6.70; found: C 57.40, H 5.26, N 6.62.

Z isomer: White solid, R_f 0.32 (heptanes-ethyl acetate 1:1), *trans*:*cis* = 28:72. M.p.= 100.5-101.5 °C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J*= 10.8 Hz, 1H, *cis*), 8.34 (s, 1H, *trans*), 7.61 (br, 1H, *cis*), 7.42 (br, 1H, *trans*), 7.12 (s, 1H, *trans*), 6.96 (s, 1H, *cis*), 6.58 (s, 1H), 6.12 (d, *J*= 2.8 Hz, 1H), 3.85 (s, 3H), 2.37 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.7 (s), 164.0 (d), 155.9 (s), 147.7 (s), 119.6 (s), 117.9 (d), 114.6 (d), 109.0 (d), 52.7 (q), 14.0 (q). MS, *m/z (%)*: 209 (M^+, 56.1%); HRMS (EI^+) for $C_{10}H_{11}NO_4$, calcd: 209.0688, found: 209.0699. Elem. Anal.: calcd: C 57.41, H 5.30, N 6.70; found: C 57.45, H 5.28, N 6.58.

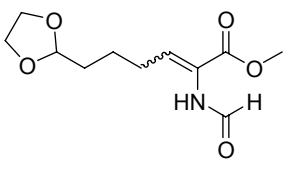
Methyl 2-(formamido)-hex-2-enoate (3.42)



E isomer: Colorless oil, R_f 0.22 (heptanes-ethyl acetate 2:1), *trans*:*cis* = 80:20.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H, *trans*), 8.24 (d, *J*= 11.1 Hz, 1H, *cis*), 7.52 (br s, 1H), 7.29 (t, *J*= 7.5 Hz, 1H, *trans*), 6.04 (t, *J*= 7.5 Hz, 1H, *cis*), 3.85 (s, 3H, *trans*), 3.83 (s, 3H, *cis*), 2.63-2.50 (m, 2H), 1.58-1.40 (m, 2H), 0.95 (dt, *J*= 7.2, 2.4 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.6 (s, *trans*), 163.8 (s, *cis*), 162.3 (d, *cis*), 159.2 (d, *trans*), 134.5 (d, *trans*), 134.1 (d, *cis*), 124.8 (s, *cis*), 123.9 (s, *trans*), 52.4 (q, *trans*), 52.3 (q, *cis*), 30.4 (t, *trans*), 30.0 (t, *cis*), 22.7 (t, *trans*), 22.6 (t, *cis*), 13.8 (q, *trans*), 13.7 (q, *cis*). MS, *m/z (%)*: 171 (M^+, 41.9%); HRMS (EI^+) for $C_8H_{13}NO_3$, calcd: 171.090, found: 171.091.

Z isomer: Colorless oil, R_f 0.19 (heptanes-ethyl acetate 2:1), *trans*:*cis* = 58:42.
^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 1H, *trans*), 8.17 (d, *J*= 11.1 Hz, 1H, *cis*), 7.20-7.00 (br, 1H), 6.75 (m, 1H, *trans*), 6.64 (m, 1H, *cis*), 3.80 (s, *trans*), 3.78 (s, *cis*), 2.32-2.08 (m, 2H), 1.60-142 (m, 2H), 1.04-0.88 (m, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.8 (s, *trans*), 164.4 (s, *cis*), 163.9 (d, *cis*), 158.9 (d, *trans*), 139.6 (d, *trans*), 135.2 (d, *cis*), 125.4 (s, *cis*), 123.2 (s, *trans*), 52.6 (q, *cis* 52.5 (q, *trans*), 31.2 (t, *trans*), 29.9 (t, *cis*), 22.1 (t, *cis*), 21.4 (t, *trans*), 13.9 (q, *trans*), 13.7 (q, *cis*). MS, *m/z (%)*: 171 (M^+, 26.9%); HRMS (EI^+) for $C_8H_{13}NO_3$, calcd: 171.090, found: 171.090.

Methyl 6-[1,3]dioxolan-2-yl-2-(formamido)-hex-2-enoate (3.43)

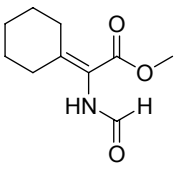


E isomer: Colorless oil, R_f 0.36 (heptanes-ethyl acetate 1:1), *trans*:*cis* = 72:28.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.30 (d, *J*= 2.0 Hz, 1H, *trans*), 8.23 (d, *J*= 10.8 Hz, 1H, *cis*), 7.49 (br, 1H, *trans*), 7.30 (t, *J*= 7.6 Hz, 1H, *trans*), 7.23 (br, 1H, *cis*), 6.02 (t, *J*= 8.0 Hz, 1H, *cis*), 4.86 (t, *J*= 4.8 Hz, 1H),

4.00-3.90 (m, 2H), 3.87-3.80 (m, 2H), 3.84 (s, 3H, *cis*), 3.82 (s, 3H, *cis*), 2.65 (q, *J*=7.6 Hz, 2H, *cis*), 2.62 (q, 7.6 Hz, 2H, *trans*), 1.75-1.56 (m, 4H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 162.1 (s), 159.1 (d), 133.7 (d, *trans*), 133.1 (d, *cis*), 124.1 (s), 104.3 (d, *trans*), 104.0 (d, *cis*), 64.8 (t, 2C), 52.5 (q), 33.4 (t, *trans*), 33.3 (t, *cis*), 28.2 (t, *trans*), 27.8 (t, *cis*), 23.8 (t, *trans*), 23.7 (t, *cis*). MS, *m/z (%)*: 243 (M^+, 16.0%); HRMS (EI^+) for $C_{11}H_{17}NO_5$, calcd: 243.111, found: 243.110.

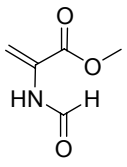
Z isomer: White solid, R_f 0.27 (heptanes-ethyl acetate 1:1), *trans*:*cis* = 58:42. M.p. 81.2-82.5 °C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H, *trans*), 8.21 (d, *J*= 11.2 Hz, 1H, *cis*), 7.18 (br, 1H, *cis*), 7.12 (s, 1H, *trans*), 6.70 (t, *J*= 7.2 Hz, 1H, *trans*), 6.57 (t, *J*= 7.6 Hz, 1H, *cis*), 4.85 (t, *J*= 4.4 Hz, 1H), 4.04-3.90 (m, 2H), 3.88-3.74 (m, 2H), 3.81 (s, 3H, *cis*), 3.78 (s, 3H, *trans*), 2.34 (q, *J*= 7.2 Hz, 2H, *cis*), 2.24 (q, J= 7.2 Hz, 2H, *trans*), 1.74-1.56 (m, 4H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 163.8 (s), 158.8 (d), 138.4 (d), 134.0 (s), 104.2 (d, *trans*), 104.1 (d, *cis*), 64.9 (t, 2C), 52.6 (q, *cis*), 52.5 (q, *trans*), 33.1 (t, *trans*), 32.6 (t, *cis*), 28.9 (t, *trans*), 27.4 (t, *cis*), 23.0 (t, *cis*), 22.5 (t, *trans*). MS, *m/z (%)*: 243 (M^+, 12.5%); HRMS (EI^+) for $C_{11}H_{17}NO_5$, calcd: 243.111, found: 243.111. Elem. Anal.: calcd: C 54.31, H 7.04, N 5.76; found: C 54.60, H 7.16, N 5.52.

Methyl 2-(formamido)-3,3-cyclohexylidene acetate (3.44)[98]



White solid, R_f 0.33 (heptanes-ethyl acetate 1:1), *trans*:*cis* ratio 65:35. M.p. 106.5-107.5 °C (Lit. 106-107 °C).
^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H, *trans*), 7.90 (d, *J*= 11.7 Hz, 1H, *cis*), 7.11 (br, 1H, *trans*), 6.90 (d, *J*= 11.1 Hz, 1H, *cis*), 3.74 (s, 3H, *cis*), 3.73 (s, 3H, *trans*), 2.78 (m, 2H, *cis*), 2.68 (m, 2H, *trans*), 2.35 (m, 2H, *cis*), 2.22 (m, 2H, *trans*), 1.62 (m, 6H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 165.2 (s), 165.1 (d, *cis*), 159.9 (d, *trans*), 155.0 (s), 152.2 (s), 117.8 (s), 116.7 (s), 52.0 (q, *cis*), 51.9 (q, *trans*), 31.7 (t, *cis*), 31.6 (t, *trans*), 30.7 (t, *cis*), 30.4 (t, *trans*), 28.2 (t, *cis*), 28.9 (t, *cis*), 27.7 (t, *trans*), 27.3 (t, *trans*), 26.0 (t). MS, *m/z (%)*: 197 (M^+, 13.3%); HRMS (EI^+) for $C_{10}H_{15}NO_3$, calcd: 197.105, found: 197.106. Elem. Anal.: calcd: C 60.90, H 7.67, N 7.10; found: C 61.00, H 7.72, N 7.07.

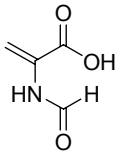
Synthesis of methyl 2-(formamido)acrylate (3.63)[99]



A mixture of serine methyl ester hydrochloric salt (1 g, 6.4 mmol), K_2CO_3 (3.5 g, 4 eq) and a catalytic amount of NEt_3 (10 µL) in methyl formate (20 mL) was stirred overnight at room temperature. The salts were filtered and the solvent removed under reduced pressure. Quick purification by column chromatography (heptanes-ethyl acetate 1:1) afforded the desired product as a white solid (92% yield). The reaction performed on a 10 gram scale afforded the product with 89% yield and the same purity.
Ratio *trans*:*cis* 88:12. R_f 0.56 (heptanes-ethyl acetate 1:1). M.p. 56.9-57.2 °C (Lit. 53-56 °C).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.56 (d, *J*= 11.4 Hz, 1H, *cis*), 8.41 (s, 1H, *trans*), 7.87 (br, 1H, *trans*), 7.60 (br, 1H, *cis*), 6.63 (s, 1H, *trans*), 5.95 (s, 1H, *trans*), 5.69 (s, 1H, *cis*), 5.44 (s, 1H, *cis*), 3.85 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 164.1 (s), 160.9 (d, *cis*), 159.4 (d, *trans*), 130.1 (s), 110.37 (t, *trans*), 104.5 (t, *cis*), 53.1 (q). MS, *m/z (%)*: 129 (M^+, 100 %); HRMS (EI^+) for $C_5H_7NO_3$, calcd: 129.043, found: 129.043. Elem. Anal.: calcd: C 46.51, H 5.46, N 10.85; found: C 46.60, H 5.51, N 10.77.

2-Formamido acrylic acid (3.77)



Prepared according to a literature procedure (16% yield).[76] White solid. Data in agreement with the literature. ^{1}H-NMR (DMSO) δ 9.59 (br s, 1H), 8.26 (s, 1H), 6.38 (s, 1H), 5.74 (s, 1H). ^{13}C-NMR (DMSO) δ 163.5, 159.9, 131.11, 107.0. HRMS (EI^+) for $C_4H_5NO_3$, calcd: 115.027, found: 115.027. Elem. Anal.: calcd: C 41.75, H 4.38, N 12.17; found: C 41.58, H 4.28, N 11.91.

Hydrogenation Procedures.

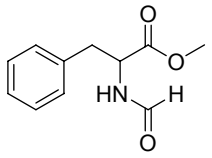
Hydrogenations were performed in an Endeavor, an autoclave with eight reactors equipped with glass reaction vessels. In a typical Endeavor run each vessel was charged open to air with $Rh(COD)_2BF_4$, monodentate phosphoramidite and substrate. Solvent was added (4 mL), the glass liners were placed in the reactors and the system was closed. After repetitive purging with N_2 (3 × 2.5 bar) the system was pressurized with hydrogen and the reactions were stirred at room temperature with 750 rpm. The conversion of the reactions was monitored by following H_2 consumption. The reactions were stopped via release of H_2 pressure. The resulting mixture was filtered over a short silica column and subjected to conversion (^{1}H-NMR and GC) and enantiomeric excess determination (capillary chiral GC).

Enantiomeric Excess Determinations.

To ensure accurate determination, all the racemic products were prepared by hydrogenation of the substrates using Pd/C (10%) and 5 bar of H_2. Absolute configuration of **3.23** was determined by comparison with the GC retention time of the commercially available enantiopure (*L*)-phenylalanine methyl ester after *N*-formyl protection. Absolute configuration of **3.74** was assigned by comparison with the sign of the literature value of the optical rotation.[100] For products **3.70**-**3.73** the absolute configurations were assigned by analogy with **3.23** and **3.74**, from the GC retention times. Substrate **3.78** was analyzed after conversion to its methyl ester (**3.74**) using (trimethylsilyl)diazomethane on a reaction sample.[101]

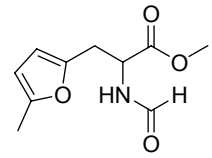
Products Data

(*R*)-Methyl 2-(formamido)-3-phenyl-propionate (3.23)[102]



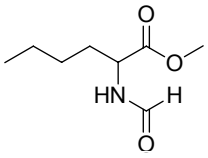
Colorless oil. Ratio *trans*:*cis* = 96:4. R_f 0.33 (heptanes-ethyl acetate 1:1).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H, *trans*), 7.66 (d, *J*= 11.6 Hz, 1H, *cis*), 7.30-7.17 (m, 3H), 7.12-7.02 (m, 2H), 6.06 (br d, *J*= 6.0 Hz, 1H, *trans*), 5.94 (br t, 1H, *cis*), 4.92 (dt, *J*= 8.0, 6.0 Hz, 1H, *trans*), 4.32-4.24 (m, 1H, *cis*), 3.73 (s, 3H, *cis*), 3.70 (s, 3H, *trans*), 3.13 (dd, *J*= 14.0, 6.0 Hz, 1H, *trans*), 3.07 (dd, *J*= 14.0, 6.0 Hz, 1H, *trans*), 2.92 (dd, *J*= 14.0, 8.4 Hz, 2H, *cis*). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 171.5 (s), 160.4 (d), 135.4 (s), 129.2 (d, 2C), 128.6 (d, 2C), 127.2 (d), 52.4 (d), 51.7 (q), 37.7 (t). MS, *m/z (%)*: 207 (M^+, 9.1%); HRMS (EI^+) for $C_{11}H_{13}NO_3$, calcd: 207.090, found: 207.091.
Enantiomeric excess determination: CP Chirasil-*L*-Val column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 160 °C, 12.5 min, 10 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 25:1; t_R = 9.61 min, t_S = 10.14 min, $t_{SM(E)}$ = 12.99 min, $t_{SM(Z)}$ = 16.06 min.

(*R*)-Methyl 2-(formamido)-3-(5-methyl-furan-2-yl)-propionate (3.70)



Colorless oil. Ratio *trans*:*cis* = 99:1. R_f 0.25 (heptanes-ethyl acetate 3:2).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H, *trans*), 7.84 (d, *J*= 12.0 Hz, 1H, *cis*), 6.33 (d br, *J*= 4.8 Hz, 1H, *trans*), 6.10 (t br, 1H, *cis*), 5.98 (d, *J*= 2.8 Hz, 1H, *cis*), 5.94 (d, *J*= 3.2 Hz, 1 H, *trans*), 5.83 (d, J= 3.2 Hz, 1H, *trans*), 4.88 (dt, *J*= 8.0, 5.2 Hz, 1H, *trans*), 4.42-4.35 (m, 1H, *cis*), 3.77 (s, 3H, *cis*), 3.75 (s, 3H, *cis*), 3.15 (dd, *J*= 15.2, 5.2 Hz, 1H, *trans*), 3.10 (dd, *J*= 15.2, 5.2 Hz, 1H, *trans*), 3.01 (dd, *J*= 15.2, 7.6 Hz, 2H, *cis*), 2.22 (s, 3H, *cis*), 2.21 (s, 3H, *trans*). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 171.2 (s), 160.5 (d), 151.4 (s), 147.8 (s), 108.8 (d), 106.1 (d), 52.5 (d), 50.1 (q), 30.5 (t), 13.4 (q). MS, *m/z (%)*: 211 (M^+, 10.4%); HRMS (EI^+) for $C_{10}H_{13}NO_4$, calcd: 211.084, found: 211.085. $[\alpha]^{20}_D$ -86.9 (*c* 1.01, $CHCl_3$), 94% ee (*R*).
Enantiomeric excess determination: CP Chirasil-*L*-Val column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 130 °C, 10 min, 5 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 25:1; t_R = 14.90 min, t_S = 15.33 min, $t_{SM(Z)}$ = 20.74 min, $t_{SM(E)}$ = 21.63 min.

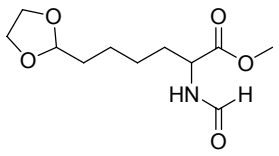
(*R*)-Methyl 2-(formamido)-hexanoate (3.71)



Colorless oil. Ratio *trans*:*cis* = 92:8. R_f 0.37 (heptanes-ethyl acetate 1:1).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H, *trans*), 8.04 (d, *J*= 12.4 Hz, 1H, *cis*), 6.18 (br, 1H, *trans*), 5.99 (br, 1H, *cis*), 4.74-4.65 (m, 1H, *trans*), 4.13-4.05 (m, 1H, *cis*), 3.76 (s, 3H, *cis*), 3.76 (s, 3H, *trans*), 1.93-1.80 (m, 1H), 1.74-1.62 (m, 1H), 1.40-1.17 (m, 4H), 0.86 (t, *J*= 7.2 Hz, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 172.6 (s), 160.5

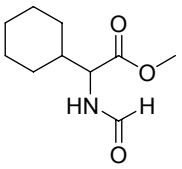
(d), 52.5 (d), 50.7 (q), 32.2 (t), 27.2 (t), 22.2 (t), 13.8 (q). MS, *m/z (%)*: 173 (M^+, 1.3%); HRMS (EI^+) for $C_8H_{15}NO_3$, calcd: 173.105, found: 173.107. $[\alpha]^{20}_D$ -36.5 (*c* 1.04, $CHCl_3$), >99% ee (*R*).
Enantiomeric excess determination: CP Chirasil-*L*-Val column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 120 °C, 20 min, 5 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 50:1; t_R = 10.50 min, t_S = 11.62 min, $t_{SM(E)}$ = 10.16 min, $t_{SM(Z)}$ = 16.08 min.

(*R*)-Methyl 6-[1,3]dioxolan-2-yl-2-(formamido)-hexanoate (3.72)



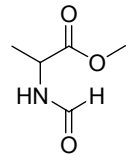
Colorless oil. Ratio *trans*:*cis* = 90:10. R_f 0.22 (heptanes-ethyl acetate 1:1).
^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1H), 6.20 (d, *J*= 6.6 Hz, 1H), 4.82 (t, *J*= 4.5 Hz, 1H), 4.69 (m, 1H), 3.93 (m, 2H), 3.84 (m, 2H), 3.75 (s, 1H), 1.86 (m, 2H), 1.68 (m, 2H), 1.40 (m, 4H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 172.5 (s), 160.6 (d), 104.3 (d), 64.8 (t, 2C), 52.5 (d), 50.7 (q), 33.4 (t), 32.3 (t), 24.9 (t), 23.4 (t). MS, *m/z (%)*: 245 (M^+, 1.6%); HRMS (EI^+) for $C_{11}H_{19}NO_5$, calcd: 245.119, found: 245.120. $[\alpha]^{20}_D$ -34.2 (*c* 1.06, $CHCl_3$), >99% ee (*R*).
Enantiomeric excess determination: CP Chirasil-*L*-Val column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 170 °C, 20 min, 5 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 50:1; t_R = 16.88 min, t_S = 17.71 min, $t_{SM(E)}$ = 17.27 min, $t_{SM(Z)}$ = 21.46 min.

(*S*)-Methyl 2-(formamido)cyclohexyl-acetate (3.73)



Colorless oil. Ratio *trans*:*cis* = 89:11. R_f 0.38 (heptanes-ethyl acetate 1:1).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H, *trans*), 7.97 (d, *J*= 11.6 Hz, 1H, *cis*), 6.31 (br d, *J*= 7.6 Hz, 1H, *trans*), 6.17 (br t, *J*= 11.2 Hz, 1H, *cis*), 4.62 (dd, *J*= 9.2, 5.2 Hz, 1H, *trans*), 3.90 (dd, *J*= 10.4, 5.6 Hz, 1H, *cis*), 3.75 (s, 3H, *cis*), 3.73 (s, 3H, *trans*), 1.86-1.52 (m, 6H), 1.32-0.97 (m, 5H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 172.1 (s, *trans*), 171.2 (s, *cis*), 163.3 (s, *cis*), 160.8 (d, *trans*), 60.0 (d, *cis*), 55.2 (d, *trans*), 52.4 (q, *cis*), 52.2 (q, *trans*), 40.91 (d, *cis*), 40.87 (d, *trans*), 29.5 (t, *cis*), 29.4 (t, *trans*), 28.0 (t, *trans*), 27.6 (t, *cis*), 25.8 (t, 3C, *trans*), 25.7 (t, *cis*), 25.6 (t, *cis*). MS, *m/z (%)*: 199 (M^+, 1.5%); HRMS (EI^+) for $C_{10}H_{17}NO_3$, calcd: 199.121, found: 199.122. $[\alpha]^{20}_D$ +46.7 (*c* 1.11, $CHCl_3$), 85% ee (*S*).
Enantiomeric excess determination: CP Chirasil-*L*-Val column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 130 °C, 10 min, 5 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 25:1; t_R = 16.84 min, t_S = 17.26 min, t_{SM} = 20.20 min.

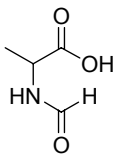
(*R*)-Methyl 2-(formamido)-propionate (3.74)[102]



Colorless oil. Ratio *trans*:*cis* = 93:7. R_f 0.23 (heptanes-ethyl acetate 1:1).
^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H, *trans*), 8.09 (d, *J*= 16.4 Hz, 1H, *cis*), 6.30 (br, 1H, *trans*), 6.12 (br, 1H, *cis*), 4.67 (m, 1H, *trans*), 4.22 (m, 1H, *cis*), 3.76 (s, 3H), 1.48 (d, *J*= 9.2 Hz, 3H, *cis*), 1.43 (d, *J*= 9.6 Hz, 3H, *trans*). MS, *m/z (%)*: 131 (M^+, 7.1%); HRMS (EI^+) for $C_5H_9NO_3$, calcd: 131.058, found: 131.059.

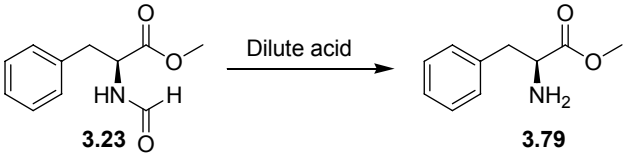
Enantiomeric excess determination: CP Chirasil-*L*-Val column (25 m × 0.25 mm × 0.25 μm). Init. Temp.: 90 °C, 10 min, 5 °C / min to 180 °C. $T_{det/inlet}$ = 250 °C, split ratio 25:1; t_R = 7.67 min, t_S = 8.71 min, t_{SM} = 5.20 min.

(*R*)-2-(Formamido)-propionic acid (3.78)



^{1}H-NMR (DMSO, *trans* rotamer) δ 8.39 (br d, *J*= 7.3 Hz, 1H), 8.00 (s, 1H), 4.30-4.22 (m, 1H), 1.25 (d, *J*= 7.3 Hz, 3H). The proton of the acid is not observed.
HRMS (EI$^+$) for $C_4H_7NO_3$, calcd: 117.0425, found: 117.0426.
Enantiomeric excess determination: after methylation providing **3.74**.

***N*-Formyl deprotection of *N*-formyl-(*L*)-phenylalanine methyl ester (3.23)**



3.23 **3.79**

Method A.[103]

Hydrochloric acid (10.0 mmol) in methanol (6 mL) was added to a solution of *N*-formyl-(*L*)-phenylalanine methyl ester (10.0 mmol) in methanol (4 mL). The reaction mixture was stirred at reflux for 1 h or at room temperature for 8 h. The reaction mixture was concentrated under reduced pressure. (*L*)-phenylalanine methyl ester hydrochloric salt was isolated after recrystallization from methanol in 99% yield.

Method B.[79a]

To a solution of *N*-formyl-(*L*)-phenylalanine methyl ester (10.0 mmol) in 2mL THF, aqueous phosphoric acid (1 mL, 85%) was added dropwise. The reaction mixture was stirred at room temperature, after 9 h 5 mL of water was added and the mixture cooled to 0 °C. An aqueous sodium hydroxide solution (10 mol/mL) was added dropwise until pH 8 was reached. The resulting mixture was extracted with ethyl acetate (3 × 20 mL) and the organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to afford (L)-phenylalanine methyl ester as colorless oil (89% yield).

3.17 References and notes

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(2) Seyden-Penne, J. *Chiral Auxiliaries and Ligands in Asymmetric Synthesis*, Wiley, New York, 1995.

(3) (a) Cleland, J. L.; Craik, C. S. *Protein Engineering: Principals and Practice*; Wiley-Liss, New York, 1996. (b) Kazmierski, W. M. *Peptidomimetics Protocols*; Humana Press, New Jersey, 1999. (c) Goodman, M.; Felix, A. *Synthesis of Peptides and Peptidomimetics*; Goodman, M. Ed.; Thieme, Stuttgart, 2003, Vol. E22b.

(4) For a recent review, see: Ma, J.-A.; *Angew. Chem. Int. Ed.* **2003**, *42*, 4290.

(5) For an overview, see Chapter 1.

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(90) (a) Ligand **A1**: Hulst, R.; de Vries N. K.; Feringa, B. L. *Tetrahedron: Asymmetry* **1994**, *5*, 699. (b) Ligands **A3 A4, A5**: Arnold, L. A.; Imbos, R.; Mandoli, A.; de Vries, A. H. M.; Naasz, R.; Feringa, B. L. *Tetrahedron* **2000**, *56*, 2865 (c) Ligand **A2**: Peña, D.; Minnaard, A. J.; de Vries, J. G.; Feringa, B. L. *J. Am. Chem. Soc.* **2002**, *124*, 14552. (d) Ligand **A6**: Duursma, A.; Lefort, L.; Boogers, J. A. F.; de Vries, A. H. M., de Vries, J. G.; Minnaard, A. J.; Feringa, B. L. *Org. Biomol. Chem.* **2004**, *2*, 1682. (e) Ligand **B4**: Bernsmann, H.; van den Berg, M.; Hoen, R.; Minnaard, A. J.; Mehler, G.; Reetz, M. T.; de Vries, J. G.; Feringa, B. L. *J. Org. Chem.* **2005**, *70*, 943. (f) Ligand **C4**: Hoen, R.; Boogers, J. A. F.; Bernsmann, H.; Minnaard, A. J.; Meetsma, A.; Tiemersma-Wegman, T. D.; de Vries, A. H. M.; de Vries, J. G.; Feringa, B. L. *Angew. Chem. Int. Ed.* **2005**, *44*, 4209.

(91) For the synthesis of PipPhos (**A4**), see: Chapter 2.

(92) Prepared according to a modified literature procedure: Ref. 46.

(93) According to a modified literature procedure: Ref. 44.

(94) Using aliphatic aldehydes, TLC (EtOAc / Heptane 1:1) analysis of the reaction mixture showed complete conversion after 2-3 hours.

(95) According to a modified literature procedure: Ref. 45c.

(96) According to a modified literature procedure: Ref. 56d.

(97) Data in agreement with the literature: Ref. 53a.

(98) Data in agreement with the literature: Yim, N. C. F.; Bryan, H.; Huffman, W. F.; Moore M. L. *J. Org. Chem.* **1988**, *53*, 4605.

(99) Data in agreement with the literature: Roos, E. C.; Lopez, M. C.; Brook, M. A.; Hiemstra, H.; Speckamp, W. N.; Kaptein, B.; Kamphuis J.; Schoemaker H. E. *J. Org. Chem.* **1993**, *58*, 3259.

(100) Chancellor, T.; Morton, C. *Synthesis* **1994**, 1023.

(101) Marcovici-Mizrahi, D.; Gottlieb, H. E.; Marks, V.; Nudelman, A. *J. Org. Chem.* **1996**, *61*, 8402.

(102) Data in agreement with the literature: Ref. 100.

(103) According to a slightly modified literature procedure: Ref. 32a.

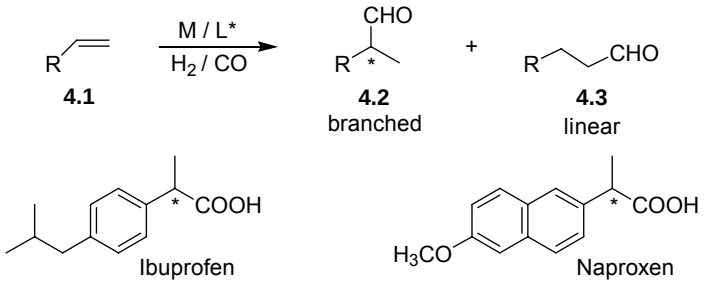
Chapter 4

Rh-Catalyzed Asymmetric Hydroformylation Using Phosphoramidite Ligands: a Preliminary Study

In this preliminary study, the feasibility of the use of monodentate phosphoramidites as chiral ligands in asymmetric Rh-catalyzed hydroformylation was established. A number of phosphoramidites with diverse backbones and/or substitutions on the amine moiety were tested in the hydroformylation of the common benchmark substrates styrene and vinyl acetate. Good activities, chemo- and regioselectivities were in general achieved. However, only modest enantioselectivities (up to 27% ee) were obtained.

4.1 Introduction

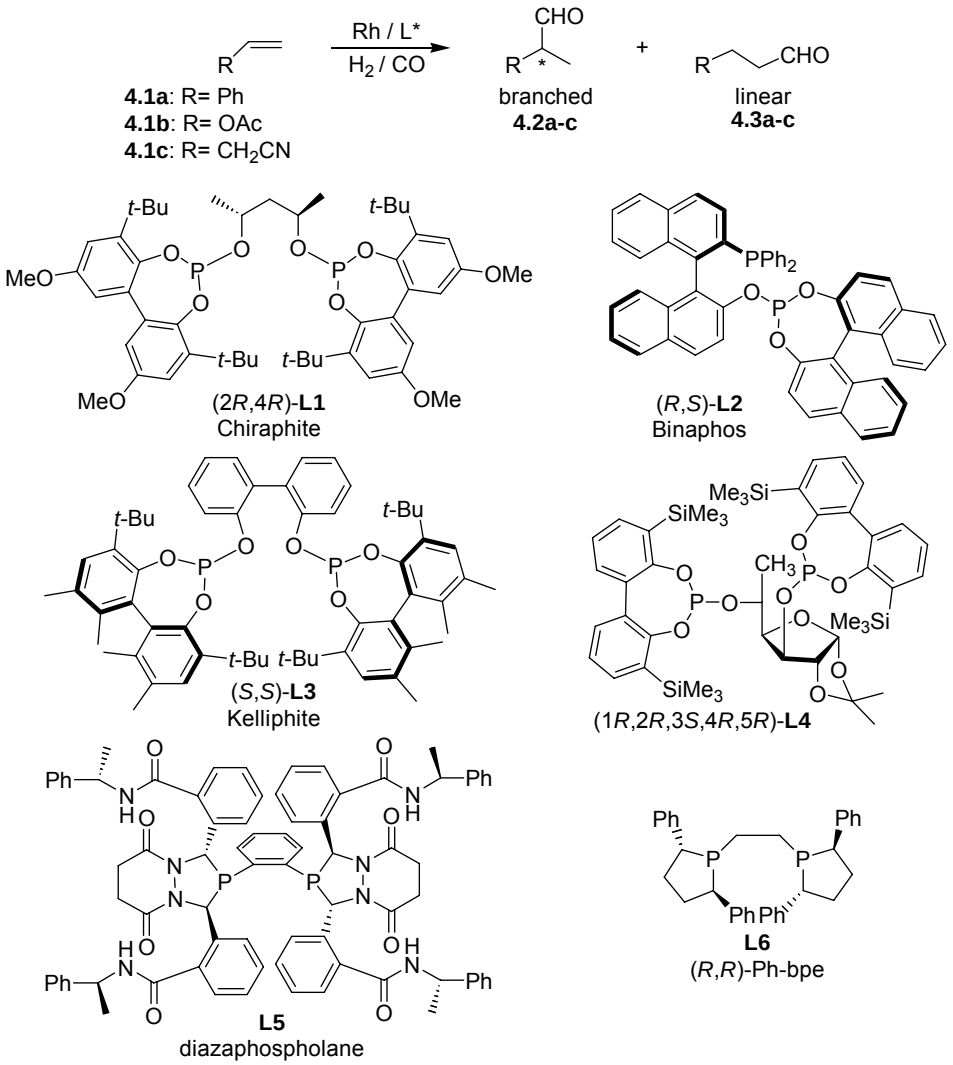
Since its discovery by Roelen in 1938,[1] the hydroformylation reaction gained an increasingly important position among industrial processes.[2] The attractive features are that a new C-C bond is formed and a new functionality is introduced at the same time, using inexpensive alkenes as starting material, syngas and a small amount of catalyst. The reaction has no equivalent in nature and it can only be performed using organometallic catalysts. Depending on the substrate used, a new stereogenic carbon may be obtained making the asymmetric hydroformylation in principle a very attractive tool for the preparation of enantiomerically enriched aldehydes (Scheme 4.1).[3] Chiral aldehydes can be precursors for a variety of pharmaceuticals and biologically active compounds (e.g. ibuprofen and naproxen), biodegradable polymers and liquid crystals.[4]



Scheme 4.1 *Asymmetric metal-catalyzed hydroformylation reactions as a source of enantiomerically enriched aldehydes*

Unlike in hydrogenation reactions, enantioselective hydroformylation catalysts need to achieve not only high levels of activity and enantioselectivity, but also induce high chemoselectivity (hydroformylation vs. hydrogenation) and regioselectivity (branched vs. linear product). In this respect, since the 1970s the best results were obtained using rhodium and platinum complexes. Using Pt-diphosphine catalysts, high enantioselectivities, but low chemo- and regioselectivities were achieved.[5] On the other hand, Rh-diphosphine catalysts showed higher activities and induced higher regioselectivities, although the enantioselectivities were modest.[6] Instead, excellent enantioselectivities and better activities have been achieved using diphosphite (up to 93% ee)[7] or phosphite-phosphine (up to 98% ee)[8] ligands. However, recently excellent results in terms of activity, regioselectivity and enantioselectivity have been achieved also using Rh-diphosphine complexes (up to 96% ee).[9]

The structure of the most successful bidentate phosphorus-based ligands **L1-6** tested in Rh-catalyzed hydroformylation of standard substrates **4.1a-c** are depicted in Scheme 4.2.



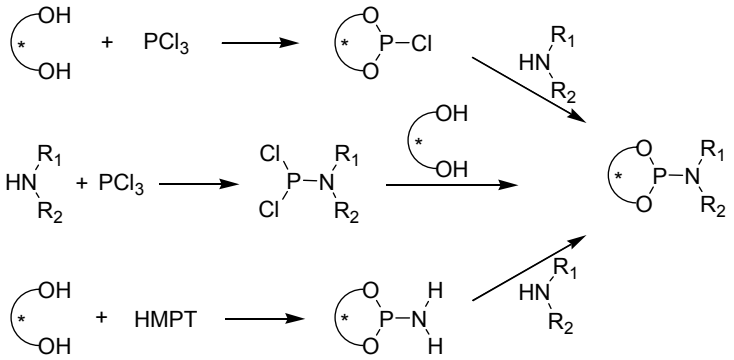
Scheme 4.2 *The most successful bidentate phosphorus based ligands*

4.1.1 Scope of this preliminary study

As previously in Rh-catalyzed asymmetric hydrogenation, the hydroformylation field is still dominated by the use of bidentate phosphorus-based ligands which provide better results in terms of reactivity and selectivity. Since 2000,[10] excellent results have also been achieved in asymmetric hydrogenation reactions using monodentate ligands.[11] Nevertheless, as far as the hydroformylation reaction is

concerned, only sporadic examples of the use of chiral monodentate ligands have been reported.

The modular structure and the ease of preparation, characteristics of monodentate ligands and in particular of the phosphoramidites developed in our group (Scheme 4.3), are extremely appealing and allow for the preparation of large libraries of ligands, which are essential for the fast identification of new and efficient chiral catalysts required for industrial applications.[12]



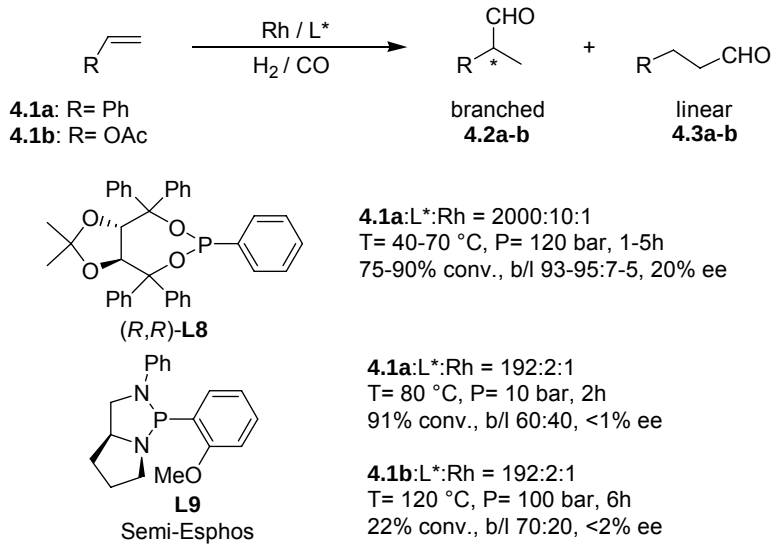
Scheme 4.3 *Modular synthesis of chiral monodentate phosphoramidite ligands*

Therefore, the scope of this preliminary study was to study the possible use of chiral phosphoramidites as ligands in the Rh-catalyzed asymmetric hydroformylation reaction, encouraged by the already excellent results obtained by this class of chiral ligands in hydrogenation reactions.[13]

4.2 Monodentate phosphorus-based ligands in Rh-catalyzed asymmetric hydroformylation

Due to low enantioselectivities and despite the good regioselectivities obtained in the early 1970's,[14] the use of monodentate chiral phosphine ligands was virtually abandoned in favor of the more efficient bidentate phosphine ligands.[15]

At the beginning of this investigation, the only more recent literature examples of chiral monodentate phosphorus ligands different than phosphines used in Rh-catalyzed hydroformylation reactions were the TADDOL-based phosphonite ligand **L8** of Seebach and coworkers[16] and the more recent diazaphospholidine **L9** of Wills and coworkers (Scheme 4.4).[17]



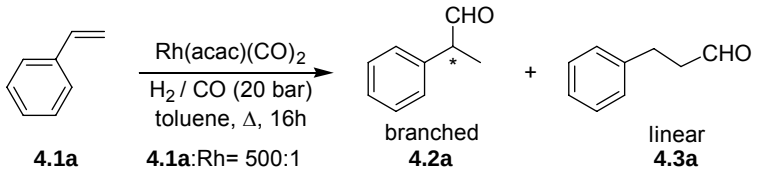
Scheme 4.4 *Chiral monodentate phosphorus-based ligands **L8-9** used in the Rh-catalyzed hydroformylation of styrene (**4.1a**) and vinyl acetate (**4.1b**)*

In the early 1990's, the monodentate TADDOL-based phosphonite **L8** was tested in a number of metal-catalyzed transformations, including the hydroformylation of styrene (**4.1a**). Only 20% enantioselectivity was induced by the catalyst and the ligand was used in excess. An excess of ligand contributes to avoid the formation of the active but not selective $RhH(CO)_3$ species caused by the dissociation or degradation of the ligand at high temperature and/or pressure, and because of the competition of CO as ligand.[18] Negligible enantioselectivities were instead obtained using ligand **L9** and the reaction conditions adopted were probably too extreme to ensure the stable coordination of monodentate ligands.

4.3 Rh-catalyzed hydroformylation of styrene (4.1a)

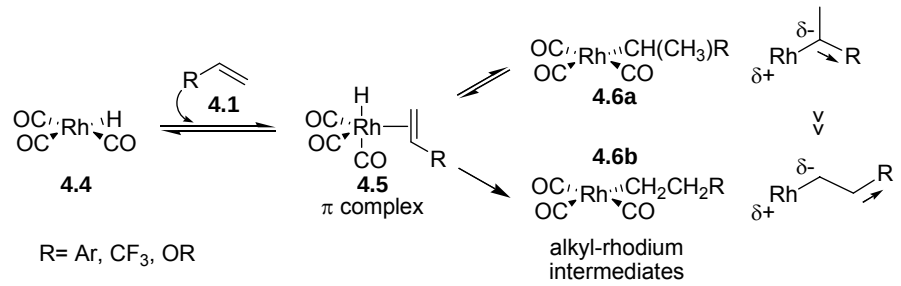
At the beginning of this investigation, the first substrate chosen was styrene (**4.1a**) as it is also the most commonly used substrate in literature reports. In order to appreciate the extent of undesired reactivity due to the formation of $RhH(CO)_3$, the hydroformylation of **4.1a** was performed in the absence of ligands using $Rh(acac)(CO)_2$ as metal precursor at two different temperatures as depicted in Scheme 4.5.

As expected, $RhH(CO)_3$, formed in situ in the presence of syngas,[18] is an active catalyst in the hydroformylation of styrene (**4.1a**) at both temperatures employed. However, at 40 °C the catalyst was less active and a higher regioselectivity in favor of the desired branched product **4.2a** was obtained.[19]



Scheme 4.5 *Hydroformylation of styrene (**4.1a**) using $RhH(CO)_3$ as catalyst*

The origin of the high regioselectivity observed, even when using an in theory unselective catalyst like $RhH(CO)_3$,can be found in the nature of the substrate and is determined in the first step of the catalytic cycle (Scheme 4.6).[20] The metal-carbon bond in the alkyl-rhodium intermediate **4.6a-b** is a polarized bond. A polarized (i.e. -Ar) or an electron withdrawing group (i.e. -OR, $-CF_3$) better delocalize the partial negative charge on the carbon. Due to inductive effects, the delocalization is better in the branched adduct than in the linear one, which causes its preferential formation.

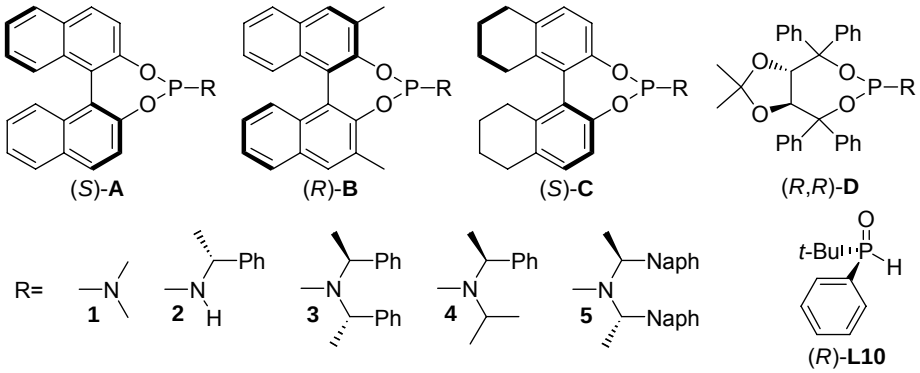


Scheme 4.6 *The first step of the catalytic cycle and the nature of the substrate **4.1** determine the regioselectivity of the products **4.2** vs. **4.3***

An increase in linear product **4.3a** at higher temperature is rationalized by the equilibrium existing between the branched alkyl-rhodium intermediate **4.6a** and the π-complex **4.5** which becomes more important at higher temperatures. As a consequence, the branched adduct **4.6a** undergoes more easily β-elimination instead of CO insertion. This equilibrium regenerates both alkyl-rhodium intermediates **4.6a-b** with a subsequent increase in linear product **4.3a**. In the hydroformylation of styrene a higher amount of branched product **4.2a** when using the unmodified rhodium precursor **4.4** as catalyst, compared to when Rh-phosphine catalysts were used, has been reported in the literature in few occasions.[20] Therefore, a low asymmetric induction and a high prevalence of **4.2a** might indicate that undesired $RhH(CO)_3$ is also present in solution.

The use of monodentate phosphorus-based ligands may induce an easier dissociation of chiral ligand in favor of CO coordination which will eventually lead to the formation of $RhH(CO)_3$. In order to limit the impact of the formation of the

unmodified Rh-complex **4.4**, it was decided to use 10 equivalents of the monodentate ligands depicted in Scheme 4.7.



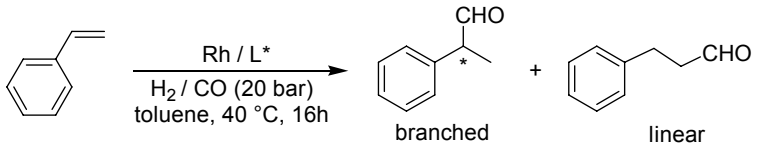
Scheme 4.7 *Monodentate BINOL- and TADDOL-based phosphoramidite ligands and phosphine oxide **L10** used in the hydroformylation of styrene (**4.1a**)*

It was decided to adopt milder reaction conditions than those reported for phosphonite **L8** or phosphine **L9** (Scheme 4.4). Rh-catalyzed hydroformylation reactions using diphosphites as chiral ligands are usually performed at moderate temperatures (25-50 °C) and syngas pressures (10-20 bar).[7] Therefore, in consideration of the similarities between phosphoramidites and phosphites, the hydroformylation of styrene was performed at 40 °C under 20 bar of syngas pressure and the results are listed in Table 4.1.

Good activities and regioselectivities were obtained in most of the cases. However, very low or no conversion was found using MonoPhos™ (**A1**) or **A2** as ligands (entries 1 and 3). An explanation for this lack of activity could be found in the use of an excess of ligand in combination with the fact that **A1** and **A2** have the smallest substituents on the amine moiety among the ligands considered. Performing kinetic studies on the Rh-catalyzed hydrogenation using MonoPhos™ (**A1**) as chiral ligand, it was discovered later that up to 4 equivalents of ligand can coordinate to the metal center.[21] Therefore, it is possible that due to the excess of ligand used, the resulting catalyst was not active anymore. Using **A1**, the small conversion obtained allowed to determine that **4.2a** had been obtained with a low enantioselectivity of 20%. An experiment was also performed at an increased temperature of 70 °C (entry 2); in this case the Rh-**A1** catalyst showed better reactivity, although lower enantioselectivity was observed (14% ee). The higher temperature applied might have favored equilibria in which different Rh-x**A1** complexes were present (due to ligand dissociation), among which the active species responsible for the reactivity observed and the lower enantioselectivity was probably caused by the increased of temperature.[22] However, the higher temperature might also have favored the formation of the active $RhH(CO)_3$, although the regioselectivity observed in favor of **4.2a** (90:10) was higher than the

one obtained under the same conditions whilst using the unmodified Rh-catalyst (67:33, Scheme 4.5), which would exclude its presence in considerable amount.

Table 4.1 *Rh-catalyzed asymmetric hydroformylation of styrene (**4.1a**) using monodentate phosphoramidite ligands*[a]



4.1a branched **4.2a** linear **4.3a**

entry	ligand	conv. (%)[b]	**4.2a**:**4.3a**	ee (%)[c]
1	(*S*)-**A1**	5	-	20
2	(*S*)-**A1**[d]	74	90:10	14
3	(*S*)-**A2**	-	-	-
4	(*S*,*S*,*S*)-**A3**	100	89:11	13
5	(*S*,*S*)-**A4**	100	90:10	8[e]
6	(*S*,*S*,*S*)-**A5**	99	93:7	2
7	(*R*,*S*,*S*)-**B3**	100	89:11	9[e]
8	(*S*,*R*,*R*)-**C3**	**89**	**94:6**	**27**[e]
9	(*R*,*R*)-**D1**	100	94:6	10[e]
10	(*R*,*R*)-**D1**[f]	100	94:6	13[e]
11	(*R*)-**L10**[g]	5	-	**31**[e]

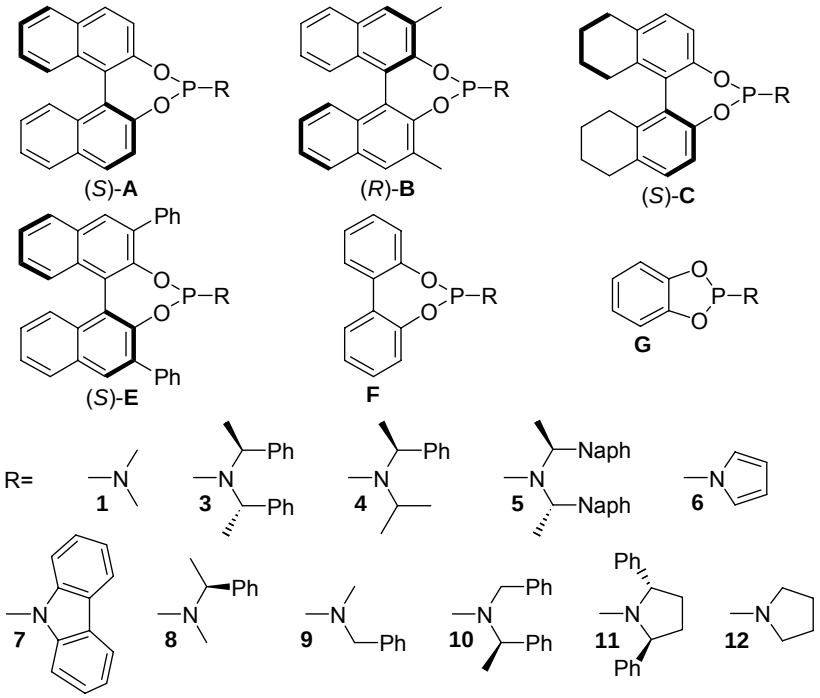
[a]Reactions performed in 2.75 mL of toluene at 40 °C and 20 bar of syngas for 16h, unless otherwise stated. Substrate:ligand:Rh = 500:10:1. The ligand and $Rh(acac)(CO)_2$ were first stirred for 1h at room temperature under an atmosphere of N_2, unless otherwise stated. [b]Conversion and b/l ratio were determined by ^{1}H-NMR and GC. No hydrogenation product was observed. [c]The enantiomeric excess was determined by chiral HPLC after reduction of the aldehyde to the corresponding alcohol, the absolute configuration was not determined. [d]Reaction performed at 70 °C. [e]The opposite enantiomer, eluting at longer HPLC elution time, was obtained. [f]The catalyst was not stirred for 1h but directly used. [g]Ligand:Rh = 14:1.

The enantioselectivities obtained were generally modest and the best compromise among reactivity, regioselectivity and enantioselectivity was found using **C4** as chiral ligand (27% ee, entry 8). The highest enantioselectivity for **4.2a** (31% ee, entry 11) was obtained by using phosphine oxide **L10** as ligand, although the activity of the catalyst was low probably due to the excess of ligand used. Nevertheless, this class of ligands might require different reaction conditions such as, for example, higher syngas pressure.[23] Interestingly, the opposite enantiomer was obtained when the configuration of either the backbone or the amine moiety

was changed (entries 7, 8 vs. 4). When a TADDOL-based phosphoramidite (**D1**) was used, the reaction was performed by first stirring the ligand and the metal precursor for 1 hour under N_2 (entry 9) or by directly transferring the complex solution into the autoclave were the reaction was performed (entry 10). Almost identical results were obtained. It is not clear whether it is actually necessary to stir ligand and metal precursor for one hour or even to pre-form the active catalyst for up to one night by stirring the complex under syngas pressure. Both protocols can be found in literature reports and the need to use either of these may mainly depend on the catalytic system used.

4.4 Rh-catalyzed hydroformylation of vinyl acetate (4.1b)

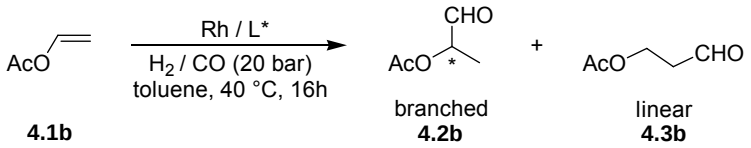
In a second set of experiments, a variety of monodentate phosphoramidite ligands were tested in the Rh-catalyzed asymmetric hydroformylation of vinyl acetate (**4.1b**). Due to the modest enantioselectivities previously obtained, the screening was extended to phosphoramidites with a larger diversity of either the backbone or the substituents on the amine moiety (Scheme 4.8).



Scheme 4.8 *Monodentate phosphoramidite ligands used in the Rh-catalyzed hydroformylation of vinyl acetate (**4.1b**)*

The reaction conditions applied were essentially the same as those used for styrene (**4.1a**). However, in view of the results obtained using Rh-**A1** as catalyst, it was decided to lower the amount of monodentate ligand to 2.5 equivalents compared to the metal precursor $Rh(acac)(CO)_2$. The results of this screening are listed in Table 4.2.

Table 4.2 *Rh-catalyzed asymmetric hydroformylation of vinyl acetate (**4.1b**) using monodentate phosphoramidite ligands*[a]



4.1b — branched **4.2b** — linear **4.3b**

entry	ligand	conv. (%)[b]	**4.2a**:**4.3a**	ee (%)[c]
1	(*S*)-**A1**	90	94:6	**17**
2	(*S*,*S*)-**A4**[d]	88	94:6	5
3	(*S*,*S*,*S*)-**A5**	100	86:14	3[e]
4	(*S*)-**A6**	**99**	**96:4**	**16**
5	(*S*)-**A7**	86	**98:2**	14
6	(*S*,*S*)-**A8**	89	90:10	15
7	(*R*,*S*,*S*)-**B3**[d]	95	93:7	8[e]
8	(*S*)-**C1**	66	88:12	9
9	(*S*)-**C9**	87	91:9	5
10	(*S*)-**E1**	31	96:4	16
11	(*S*,*S*)-**G3**	97	90:10	3[e]
12	(*S*)-**G10**	82	94:6	3
13	(*R*,*R*)-**G11**	92	96	4

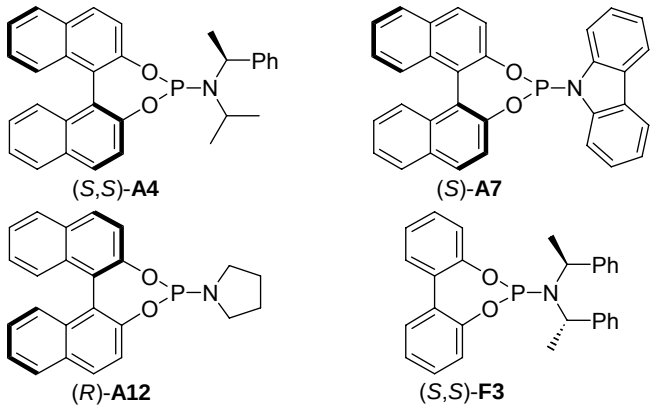
[a]Reactions performed in 3 mL of toluene at 40 °C and 20 bar of syngas for 16h. Substrate:ligand:Rh = 500:2.5:1, unless otherwise stated. The ligand and $Rh(acac)(CO)_2$ were first stirred for 1h at room temperature under an atmosphere of N_2. [b]Conversion and b/l ratio were determined by ^{1}H-NMR. No hydrogenation product was observed. [c]The enantiomeric excess was determined by chiral GC, the absolute configuration was not determined. [d]Ligand:Rh = 3:1. [e]The opposite enantiomer, with higher GC retention time, was obtained.

Rather good reactivities and regioselectivities were achieved in almost all cases. However, **4.2b** was obtained with low enantioselectivities. Only 18% conversion to the products was observed performing an experiment under the same conditions, but in the absence of chiral ligand. The unmodified catalyst **4.4** was, therefore, less

active in the hydroformylation of **4.1b**, which is important as a lower ligand to metal ratio was used. The use of MonoPhos™ (**A1**) provided good activity and the most selective catalyst (17% ee, entry 1), confirming that an excess of ligand leads to an inactive catalyst at least when using phosphoramidite ligands with a small amine moiety (entries 1 and 3, Table 4.1).

The presence of a chiral backbone seems to be important as the cathecol-based phosphoramidites provided catalysts with low enantioselectivities (entries 11-13).[24] The best compromise among reactivity, regioselectivity and enantioselectivity was achieved using the Rh-**A6** complex (entry 4), but the best regioselectivity was obtained using **A7** as chiral ligand (entry 5). The catalyst resulting from the use of **E1** as ligand yielded **4.2b** again with 16% ee, although it was less active, as only 31% conversion to the products was observed (entry 10). The bulkiness of the backbone of the ligand might be responsible for the lower reactivity, as regioselectivity and enantioselectivity were identical to those obtained using **A1**, which bears the same amine moiety. The presence of bulky chiral amine substituents led to less selective catalysts (**A4**, **A5**, and **B3**, entries 2, 3 and 7).

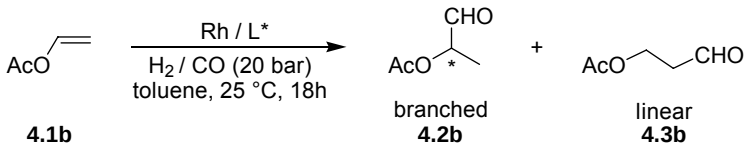
A small selection of monodentate phosphoramidites (Scheme 4.9) were again tested in the hydroformylation of **4.1b**. The reactions were run under essentially the same conditions but at room temperature, in order to see if it was possible to achieve higher enantioselectivities.



Scheme 4.9 *Monodentate phosphoramidite ligands tested in the Rh-catalyzed hydroformylation of vinyl acetate (**4.1b**) at room temperature*

Ligands **A4** and **A7** were previously tested at 40 °C (entries 2 and 5, Table 4.2), ligands **A12** and **F3**, instead, had not been used before. The results obtained are depicted in Table 4.3 and, as expected, at room temperature the catalysts showed very little reactivity. A good 48% ee was obtained using **A7** as chiral ligand (entry 3) compared with the 14% ee obtained at 40 °C, although at very low conversion.

Table 4.3 *Rh-catalyzed asymmetric hydroformylation of vinyl acetate (**4.1b**) performed at room temperature*[a]



4.1b | branched **4.2b** | linear **4.3b**

entry	ligand	conv. (%)[b]	ee (%)[c]
1	-	<1	-
2	(*S*,*S*)-**A4**	14	11
3	(*S*)-**A7**	2	**48**
4	(*R*)-**A12**	1	11[d]
5	(*S*,*S*)-**F3**	23	4

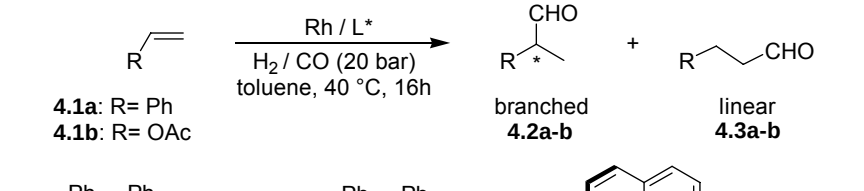
[a]Reactions performed in 3 mL of toluene at room temperature and 20 bar of syngas for 18h. Substrate:ligand:Rh = 500:3:1. The ligand and $Rh(acac)(CO)_2$ were first stirred for 1h at room temperature under an atmosphere of N_2. [b]Conversions were determined by ^{1}H-NMR. Due to the low conversions, the b/l ratio was not determined. No hydrogenation product was observed. [c]The enantiomeric excess was determined by chiral GC. [d]The opposite enantiomer, with higher GC retention time, was obtained.

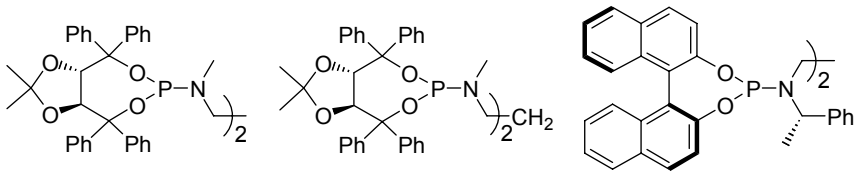
The use of ligands bearing BINOL moieties with opposite configuration but different amine groups afforded opposite enantiomers of **4.2b** with identical enantioselectivities (entries 2 and 4). Moreover, a less selective even if more reactive catalyst was obtained using **F3** (entry 5), whose backbone is instead not chiral. Also these data seem to confirm that the use of chiral amines has less impact on the enantioselectivity than the presence of a chiral diol backbone.

4.5 Bidentate phosphoramidite ligands in the Rh-catalyzed hydroformylation of styrene (4.1a) and vinyl acetate (4.1b)

In order to understand if the modest enantioselectivities obtained were intrinsically caused by the use of monodentate ligands, a couple of experiments were performed using bidentate phosphoramidites in the Rh-catalyzed hydroformylation of both styrene (**4.1a**) and vinyl acetate (**4.1b**).

Table 4.4 *Rh-catalyzed asymmetric hydroformylation of styrene (**4.1a**) and vinyl acetate (**4.1b**) using bidentate phosphoramidite ligands*[a]





(*R*,*R*,*R*,*R*)-**L11** (*R*,*R*,*R*,*R*)-**L12** (*S*,*S*,*S*,*S*)-**L13**

entry	ligand	L*:Rh(I)	substrate	conv. (%)[b]	**4.2**:**4.3**	ee (%)[c]
1	**L11**	2.5:1	**4.1a**	82	86:14	3
2	**L12**	2.5:1	**4.1a**	100	88:12	2[d]
3	**L13**	1.2:1	**4.1b**	74	89:11	**27**

[a]The ligand and $Rh(acac)(CO)_2$ were first stirred for 1h at room temperature under an atmosphere of N_2. Substrate:Rh = 500:1. [b]Conversion and b/l ratio were determined by ^{1}H-NMR and GC. No hydrogenation product was observed. [c]The enantiomeric excess was determined by chiral GC or chiral HPLC. [d]The opposite enantiomer, with higher GC retention time, was obtained.

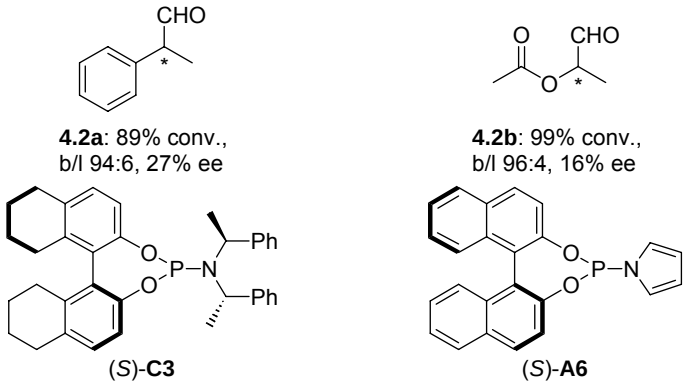
The use of two differently bridged bidentate TADDOL-based phosphoramidites **L11** and **L12** did not provide better catalysts for the hydroformylation of styrene (**4.1a**) as **4.2a** was obtained with lower regioselectivities and negligible enantioselectivities (entries 1 and 2) compared to the best results so far obtained using **C3** (94:6, 27% ee, Table 4.1). On the other hand, the catalyst obtained using the BINOL-based phosphoramidite **L13** provided the best enantioselectivity so far obtained in this study in the hydroformylation of vinyl acetate (**4.1b**) reaching 27% ee (entry 3) and the regioselectivity found was similar to the one observed using its monodentate equivalent **A8** (90:11, Table 4.3). Nevertheless, the enantioselectivity achieved is rather modest for a bidentate ligand as **4.2b** was obtained in 96% ee using the bidentate diazaphospholane **L5** reported by Landis and coworkers (Scheme 4.2).[9a]

4.6 Conclusions and outlook

At the end of this preliminary investigation, the conclusion is that monodentate phosphoramidites are suitable chiral ligands for Rh-catalyzed hydroformylation reactions. Good results at least in terms of chemo- and regioselectivity were

obtained. Nevertheless, the low enantioselectivities obtained demonstrate that further research needs to be done in order to achieve satisfactory performances and transform monodentate ligands in a competitive and desirable choice.

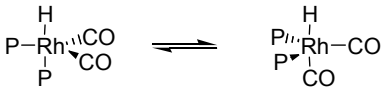
Summarizing the results obtained in the hydroformylation of the standard substrates **4.1a-b** (Scheme 4.10), it is possible to notice that a rather different phosphoramidite ligand, among those tested, was found to be more *effective* in each case.



Scheme 4.10 *Different monodentate phosphoramidites resulted to be more suitable ligands for each of the standard substrates **4.1a-b***

The different substrates might have different spatial requirements, therefore, ligands with different substitutions might be necessary to achieve better performances; nevertheless, these results show that the best ligand has not been found yet and the screening of a larger number of phosphoramidites using a large diversity of backbone structures and substitutions on the amine group should be performed. The reaction conditions adopted might also not be the most suitable for this class of ligands. For example, in Rh-catalyzed hydrogenation reactions using monodentate phosphoramidites as ligands it was found that the best solvent was not the most commonly used MeOH but CH_2Cl_2 instead.[25]

As the results obtained using monodentate ligands in terms of enantioselectivity are still rather poor, it might also be useful to reconsider the catalytic system and the structural characteristics of the Rh-complex. It is well known that the resting state of the hydroformylation catalyst is a trigonal bipyramidal hydrorhodium carbonyl $RhH(CO)_2(PP)$ complex. Ideally, in order to reach high enantioselectivities, the resting state of the catalyst should be conformationally stable. However, it is also well known that this complex can exist as a mixture of two isomers in which the two phosphorus ligands are in equatorial-apical (**ea**) or equatorial-equatorial (**ee**) positions as depicted in Scheme 4.11.[26]



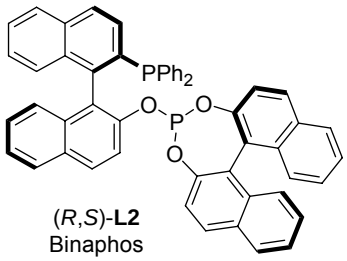
ea equatorial-apical **ee** equatorial-equatorial

Scheme 4.11 *The resting state of the hydroformylation catalyst [RhH(CO)$_2$(PP)] can be present as a mixture of two isomers.*

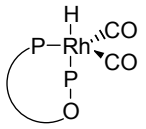
Experimental observations reported in the literature seem to correlate the degree of enantioselectivities obtained with the degree of preference of one isomer over the other.[27] Moreover, increasing temperatures and syngas pressure might favor a rapid equilibrium between the two isomers, even of complexes that at room temperature seem to be conformationally stable.

The structure of the ligand and the nature of the phosphorus moiety might also influence the predominance of one isomer over the other. For example, a phosphine, being a better σ-donor, should preferably coordinate in apical position. A phosphite, which is a better π-acceptor, should preferably coordinate in equatorial position.[28] In case of bidentate ligands with two identical phosphorus groups, the isomeric ratio is influenced by the P-M-P bite angle, as bidentate ligands with 90° bite angle will adopt an equatorial-apical conformation, whereas with bite angles of around 120° an equatorial-equatorial conformation is instead preferred. Numerous studies have been devoted to the design of ligands with different bite angles because of their influence on the regioselectivities.[29]

The most efficient and versatile chiral bidentate ligand to date is Binaphos (**L2**) with enantioselectivities ≥90% for a variety of substrates.[8] The structure of this phosphine-phosphite based ligand is depicted in Scheme 4.12. It combines the higher catalytic activities showed by Rh-diphosphines complexes[30] and the higher enantioselectivities obtained with Rh-diphosphite complexes, which nevertheless have limited scope.[7a,b]



(*R*,*S*)-**L2**
Binaphos



No equatorial-apical exchange
at either 60 °C or 25 °C

Scheme 4.12 *The most efficient and versatile chiral bidentate ligand: Binaphos (**L7**)*

Contrary to what was generally believed at that time, Binaphos (**L2**) is a clear example that it is not necessary to use C_2 symmetric ligands to obtain high enantioselectivities.[31] This was considered essential in order to limit the amount of

catalytically active species present during catalysis. The real strength of this chiral ligand appears to be the extreme conformational stability of the corresponding Rh-complex even under reaction conditions and at different temperatures, which implies that only one complex is present in the reaction mixture. Nevertheless, the Rh-Binaphos complex has the phosphine group in equatorial position and the phosphite in apical position which is in disagreement with what expected according to the electronic properties of the phosphorus groups. This phenomenon has still no explanation.

Conformational stability of the Rh-complex is therefore extremely important, but clearly much more difficult to achieve using monodentate ligands. The studies conducted by Brown and Kent showed that there is a 85:15 **ee**/**ea** mixture of isomers for the $RhH(CO)_2(PPh_3)_2$ complex in fast exchange even at room temperature.[32] The use of monodentate ligands might also favor an easier dissociation of the ligand from the complex and more than two equivalents of ligand might be coordinated if an excess of ligand is used, which on the other hand might be necessary to avoid the formation of $RhH(CO)_3$. Under these conditions, many species might be present in solution during the entire catalytic cycle.

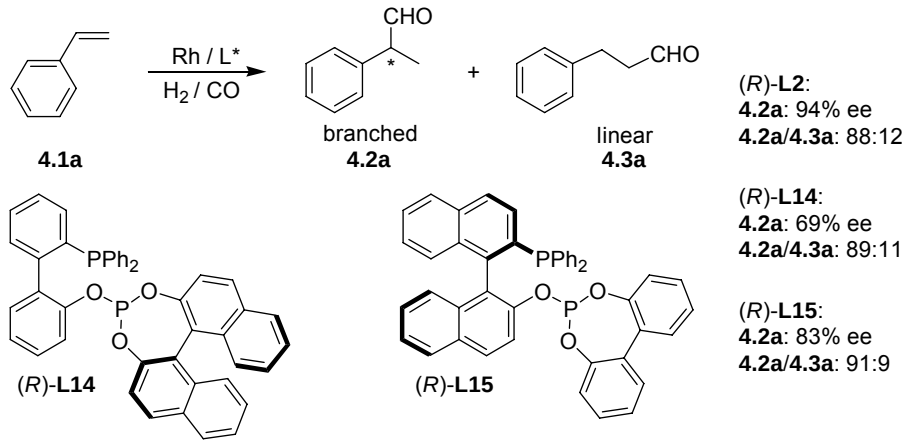
The developments of Rh-phosphoramidite complexes with the structural similarity to those based on bidentate ligands could be pursued in two different ways:

1. Using a homo-complex of two phosphoramidites ligands with either a bulkier backbone or amine moiety.

The right degree of steric bulk might force a preferential equatorial-equatorial or equatorial-apical conformation. In principal, either of the two isomers could induce high enantioselectivities as long as predominant and stable under the reaction conditions. Bulkiness on both sides might instead favor complex instability and dissociation.

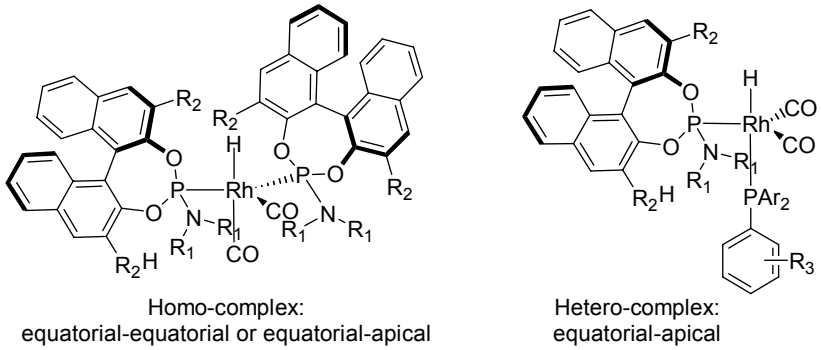
2. Using a hetero-complex of two different types of phosphorus ligands, such as phosphoramidites and phosphines.[33,34]

Assuming that phosphoramidite ligands behave similarly to phosphite ligands, the different electronic properties of the phosphorus atoms should provide hetero-complexes which might have the characteristic equatorial-apical preference showen by Binaphos. However, a study conducted on modifications of the structure of Binaphos revealed that the presence of only one chiral moiety in the ligand provided lower enantioselectivities (Scheme 4.13).[8b] This means that both ligand substitutions both on the backbone as well as on the amino group might be important in order to regain the enantioselectivity which might be lost because of the use of an achiral phosphine.



Scheme 4.13 *Influence of the backbone structure on the enantioselectivity in the hydroformylation of styrene (**4.1a**)*

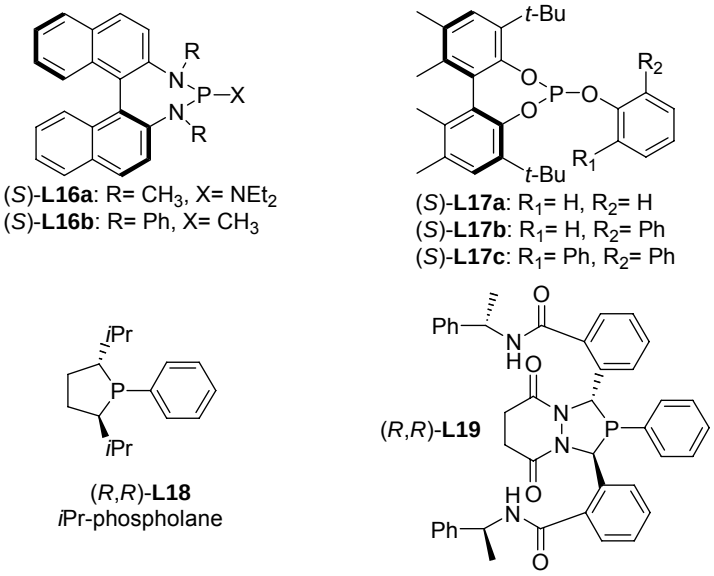
Both types of Rh-complexes can be easily investigated due to the modular structure of the monodentate phosphoramidites allowing a combinatorial approach to the easy preparation of large libraries of chiral ligands.



Scheme 4.14 *Possible structures of the homo- and hetero-complexes*

4.7 Further developments

Since our investigation, a few other chiral monodentate phosphorus-based ligands, which appeared in the literature, (Scheme 4.15) have been studied and the best results obtained in the hydroformylation of the standard substrates **4.1a-c** are depicted in Table 4.5.[35]



Scheme 4.15 *New chiral monodentate phosphorus-based ligands used in the Rh-catalyzed asymmetric hydroformylation of alkenes **4.1a-c***

As shown in Table 4.5, the conditions applied in the various systems are rather different in terms of catalyst loading, temperature, pressure and reaction time with consequent influence on the reaction outcome. In all cases, the ratio between monodentate ligands and rhodium precursor was lowered to values between 3.0 and 2.1. Reaction rates in hydroformylation are generally lower than in hydrogenation reactions, which are often performed at room temperature and low H_2 pressure (1-5 bar H_2), therefore higher temperature and syngas pressure are required. Nevertheless, higher temperatures and/or syngas pressures might induce lower enantioselectivities and worse ratios between the desired branched **4.2a-c** and the linear **4.3a-c** products, which make it difficult to compare the various catalysts in terms of regio- and enantioselectivity.

The enantioselectivities obtained are still rather modest compared to what obtained using bidentate ligands (see Chapter 1). For example, ligands **L17a-c**[35c] and **L19**[35d] were used as monodentate equivalents of the corresponding bidentate ligands (**L3** and **L5**, Scheme 4.2) which under the same conditions afforded enantioselectivities up to 96% ee. The best results in terms of enantioselectivities in the hydroformylation of styrene (**4.1a**, up to 37% ee) and allyl cyanide (**4.1c**, up to 43% ee) were obtained using diazaphospholidines **L16a-b**[35b] and phosphites **L17a-c**[35c] (entries 1-7). The Rh-phosphite complexes reported by Whiteker and coworkers showed the highest activities as milder conditions could be used (10 bar syngas at 35 °C). The milder conditions applied allowed using acetone as a solvent instead of the most commonly used toluene. However, it is difficult to conclude if this had any influence on the results, as no systematic study has been performed.

Table 4.5 *Rh-catalyzed asymmetric hydroformylation of alkenes **4.1a-c** using monodentate chiral ligands **L16-19***[a]

4.1a: R= Ph
4.1b: R= OAc
4.1c: R= CH_2CN

branched **4.2a-c** | linear **4.3a-c**

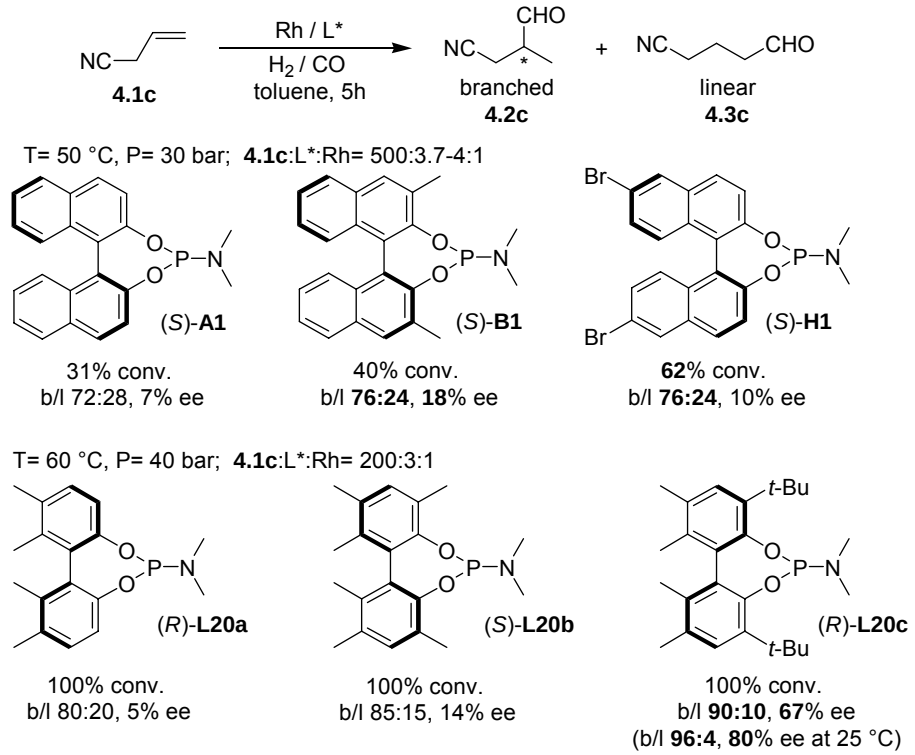
entry	ligand[b]	substrate[c]	T (°C)	P (bar)	conv. (%)	**4.2**:**4.3**	ee (%)
1	**L16a** (3)	**4.1a** (400)	60	50	100 (20h)	78:22	**37**
2	**L16b** (3)	**4.1a** (400)	25	25	98 (20h)	80:20	**31**
3	**L17a** (2.2)	**4.1a** (300)	35	10	43 (3h)[d]	**94:6**	**37**
4	**L17a** (2.2)	**4.1c** (300)	35	10	61 (3h)[d]	**84:16**	**43**
5	**L17b** (2.2)	**4.1a** (300)	35	10	59 (3h)[d]	94:6	**28**
6	**L17b** (2.2)	**4.1c** (300)	35	10	87 (3h)[d]	83:17	**37**
7	**L17c** (2.2)	**4.1c** (300)	35	10	23 (3h)[d]	83:17	13
8	**L18** (2.1)	**4.1a** (5000)	80	13	11 (3h)	81:18	11
9	**L18** (2.1)	**4.1b** (5000)	80	13	17 (3h)	**95:5**	8
10	**L18** (2.1)	**4.1c** (5000)	80	13	30 (3h)	78:12	7
11	**L19** (2.1)	**4.1a** (5000)	80	10	28 (3h)	52:48	12
12	**L19** (2.1)	**4.1b** (5000)	80	10	31 (3h)	**98:2**	**23**
13	**L19** (2.1)	**4.1c** (5000)	80	10	93 (3h)	57:43	10

[a]Reactions performed in toluene unless otherwise stated. [b]Equivalents of ligand used compared to $Rh(acac)(CO)_2$. [c]Equivalents of substrate used compared to $Rh(acac)(CO)_2$. [d]Reactions performed in acetone.

Vinyl acetate (**4.1b**) is a particularly difficult substrate (entries 9 and 12) and also the less studied one. Besides the low enantioselectivities obtained, very good regioselectivities toward the branched product **4.2b** were reported using the phosphine-based ligands **L18**[35e] and **L19** (entries 9 and 12).

4.7.1 New studies on the use of monodentate phosphoramidite ligands[36]

The use of monodentate phosphoramidite ligands has been further investigated first by De Vries and Lambers-Verstappen[37] and subsequently by Ojima and coworkers (Scheme 4.16).[38] Both classes of phosphoramidite ligands were tested in the hydroformylation of allyl cyanide (**4.1c**).



Scheme 4.16 *Rh-catalyzed asymmetric hydroformylation of allyl cyanide **4.1c** using monodentate phosphoramidites as chiral ligands*

Whilst using BINOL-based monodentate phosphoramidite ligands **A1**, **B1** and **H1**[37] good results in terms of reactivity and regioselectivity were achieved in the hydroformylation of **4.1c**, with Rh-**H1** being the best catalyst affording 62% conversion to the products with a 76% preference for the branched product **4.2c**. Nevertheless, also for this substrate, the catalysts provided by the coordination of $Rh(acac)(CO)_2$ with the phosphoramidite ligands tested was not very enantioselective. The best result was obtained using **B1** as chiral ligand and **4.2c** was obtained with 18% ee, which suggested that ligands with substituents in the 3- and 3'-position could lead to more enantioselective catalysts. The results, at least in terms of enantioselectivities, appeared to be in line with those obtained during the investigation described in this chapter. However, the highest enantioselectivity using **4.1c** was achieved once more using a different ligand (Scheme 4.10).

The best results reported to date for chiral monodentate ligands were achieved using the chiral bisphenol-based phosphoramidites **L20a-c** reported one year later by Ojima and coworkers.[38] The ligand screening was performed at 60 °C and using 40 bar of syngas, **4.3c** was obtained with up to 67% ee using the bulkier ligand **L20c**. The results obtained using **L20a** and **L20b** are similar to those reported

using the corresponding BINOL-based **A1** and **B1**, confirming the importance of the substitution in 3- and 3'- positions. A temperature screening provided 80% ee (25 °C) and an increased regioselectivity of 96:4 in favor of **4.3c**, although complete conversion in this case was reached after only 74h. Contrary to what has been postulated in this chapter (page 14), a solvent screening confirmed toluene to be the best solvent among those tested (THF, DCM, MeOH), although acetone was not tested.

Trying to understand the reasons behind the dramatic effect observed by using the bulkier ligand **L20c**, the authors performed also a molecular modeling study, which showed that due to steric repulsion the two ligands were coordinated in the equatorial-equatorial positions of the trigonal-bipyramidal Rh-complex (see for example the homo-complex in Scheme 4.14). Further investigations into the scope of the reaction using this ligand (or other with similar substitution patterns) should be performed.

4.8 Experimental section

General Procedures.

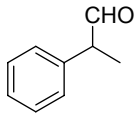
For general remarks, see Chapter 2.

Ligands **A1**,[39] **A12**[40] and **C1**[25] were kindly provided by Michel van den Berg. Ligand **A2** was kindly provided by Adri Minnaard and Diego Peña.[41] Ligands **A6-9** were kindly provided by Jean-Guy Boiteau.[42] Ligands **G3** and **G10**-**11** were kindly provided by Rob Hoen.[24] Ligand **L10** was kindly provided by Xiao-Bin Jiang.[23] All the other mono- and bidentate phosphoramidite ligands were kindly provided by Roos Imbos and Leggy Arnold.[43]

Hydroformylation Procedure.

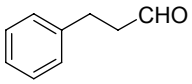
In a typical experiment, $Rh(acac)(CO)_2$ was added as a solution in distilled toluene (1.5 mL, 6.5 μmol) to a flame dried glass vial containing the desired amount of chiral ligand. The resulting solution was stirred at room temperature under an atmosphere of N_2 for one hour. The substrate (styrene was usually distilled and stored at 4 °C) was subsequently added as a toluene solution (1.5 mL, 3.25 mmol). The glass vial was transferred to an autoclave which was previously dried under reduced pressure for one hour. After purging with N_2 (3 × 5 bar) the system was heated to the desired temperature and pressurized with syngas (H_2:CO, 1:1) and the reaction mixture was stirred for 16 h. The reaction was stopped by first cooling the autoclave to room temperature and then releasing the syngas pressure. A sample of the reaction mixture was analyzed by ^{1}H-NMR and chiral GC or HPLC for conversion and enantiomeric excess.

2-Phenylpropanal (4.2a)



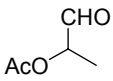
^{1}H-NMR (300 MHz, CDCl3) δ 9.70 (d, *J*= 2.1 Hz, 1H), 7.43-7.12 (m, 5H), 3.64 (q, *J*= 10.5 Hz, 1H), 1.45 (dt, *J*= 10.5, 2.1 Hz, 3H). Enantiomeric excess determination: A sample of the organic solution (300 µL) was added to EtOH (2 mL) followed by an excess of $NaBH_4$ (a spatula tip) and the heterogeneous solution was stirred for 90 min. at room temperature.[44] The reaction was quenched by adding H_2O (3 mL) and the mixture was extracted with EtOAc/hexane (1:1, 3 times). The combined organic layers were dried over Na_2SO_4 and the solvent removed. A sample of the crude mixture (100 µL) was added to a solution of hexane and isopropanol (90:10). A sample of the solution obtained (5-10 µL) was used for the determination of the enantiomeric excess by HPLC. Conditions: OB-H column; heptane/isopropanol, 975:25; 0.5 mL/min, λ_{det} 220 nm. Retention time: 2-phenyl-propan-1-ol (from **4.2a**), 17.0/18.3 min.; 3-phenyl-propan-1-ol (from **4.3a**), 20.4 min.

3-Phenylpropanal (4.3a)[45]



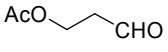
^{1}H-NMR (300 MHz, CDCl3) δ 9.83 (t, *J*= 1.5 Hz, 1H), 7.35-7.10 (m, 5H), 2.96 (t, *J*= 7.5 Hz, 2H), 2.77 (tt, *J*= 7.5, 1.5 Hz, 2H).

2-Acetoxypropanal (4.2b)



^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.44 (d, *J*= 1.5 Hz, 1H), 4.97 (dq, *J*= 7.2, 1.5 Hz, 1H), 2.08 (d, *J*= 2.1 Hz, 3H), 1.31 (dd, *J*= 7.2, 2.1 Hz, 3H). Enantiomeric excess determination: Chiraldex β-TA column (30 m × 0.25 mm × 0.125 µm). Init. Temp.: 80 °C, 30 min, 10 °C / min to 140 °C. $T_{det/inlet}$ = 200 °C, split ratio 100:1; $t_{R/S}$ = 9.80/31.74 min, t_{SM} = 2.47 min.

3-Acetoxypropanal (4.3b)[46]



^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.65 (d, *J*= 2.1 Hz, 1H), 4.30 (dt, *J*= 6.0, 2.1 Hz, 2H), 2.67 (tt, *J*= 6.0, 1.8 Hz, 2H), 1.95 (d, *J*= 1.8 Hz, 3H).

4.9 References and notes

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Chapter 5
Merging Homogeneous Catalysis with Biocatalysis: Papain as Hydrogenation Catalyst

Papain, modified at Cys-25 with a monodentate phosphite ligand and complexed with $Rh(COD)_2BF_4$, is an active catalyst in the hydrogenation of methyl 2-acetamidoacrylate.

[…] the pace, at which 'new' enzymes for selective biotransformations are appearing, is astonishingly modest. The reasons for this are probably manifold: first, the conservatism of academic and industrial researchers avoiding *high-risk projects*; second, lack of *interdisciplinarity* among chemists and biologists; […].

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5.1 Introduction

There are three main routes for the preparation of enantiomerically pure compounds: (1) separation of racemates; (2) transformation of a precursor provided by natural sources from fermentation or agriculture; (3) asymmetric synthesis from prochiral substrates using both catalytic and stoichiometric methods (Figure 5.1).

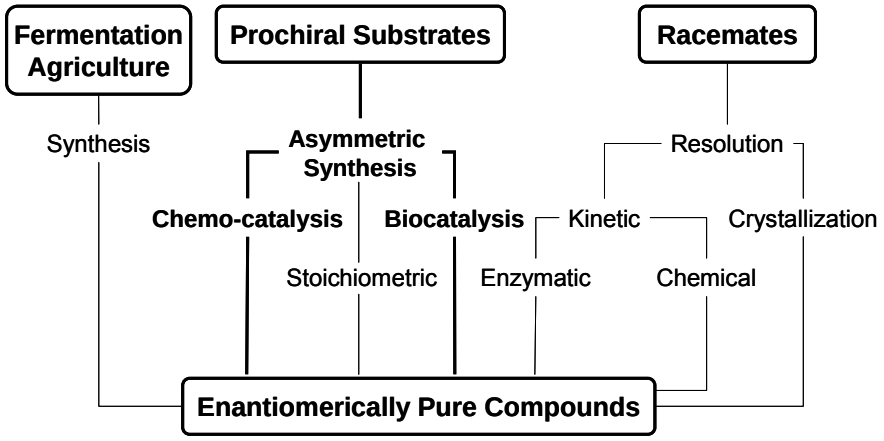
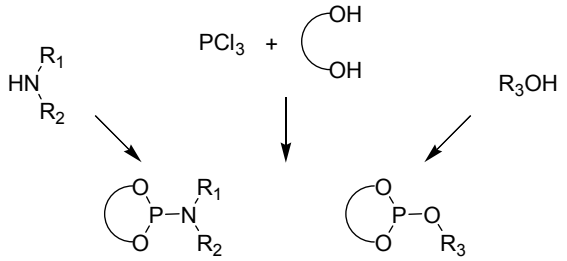


Figure 5.1 *Routes to enantiopure compounds*

The most convenient and attractive of these methodologies, in terms of atom efficiency and mildness of reaction conditions, are enantioselective chemo-catalysis and biocatalysis.

Enantioselective metal-catalyzed transformations have developed enormously in the last decades, providing fundamental contributions to the establishment of modern organic chemistry.[1] Nevertheless, although homogeneous asymmetric catalysis has reached an advanced stage in the laboratory, the method of choice to obtain enantiopure intermediates, in the production of pharmaceuticals, is still the resolution by crystallization of diastereomeric salts.[2] One of the main reasons for this is the strong time-to-market pressure related to their production. Fortunately, high throughput experimentation can be used increasingly for the rapid identification of a chiral catalyst.[3] The availability of large libraries of chiral catalysts and in particular of the corresponding ligands can be, however, a serious bottleneck.[4] Phosphorus based chiral ligands are arguably the most versatile ligands for asymmetric catalysis. However, the availability of chiral bidentate phosphorus-based ligands is often associated with cumbersome and lengthy synthesis. Recently, there has been a revival of the use of monodentate chiral phosphorus ligands and excellent results have been achieved in different fields of asymmetric catalysis.[5] Moreover, monodentate phosphoramidites, developed in

our laboratories, and monodentate phosphite ligands have the advantage of a simple modular design which allows the versatile synthesis of a large diversity of structures (Scheme 5.1).[6] These ligands can be synthesized in an automated manner, opening the doors to large libraries and truly combinatorial approaches.[7]



Scheme 5.1 *Modular structure of phosphoramidites and phosphites*

Biocatalysis is also increasingly applied in organic chemistry for a variety of transformations, emerging as a valuable alternative for the preparation of enantiopure compounds.[8] Characteristics such as chemo-, regio- and stereoselectivity manifested by biocatalysts are among the most appealing features connected with their use. Nevertheless, also in this field, despite the continuous efforts, a limited amount of industrial applications has been established.[2b,9] Some of the reasons attributed to the limited implementation of biocatalysis into production seem to be: (1) the commercial availability of the biocatalysts; (2) their limited substrate scope; (3) their operational stability; (4) the reluctance of the chemical community to fully explore their potential.[10]

Inevitably, chemocatalysis and biocatalysis have fundamental differences in their positive and negative features (some of which are listed in Table 5.1) that make them rather complementary.

Table 5.1 *Characteristics of homogeneous and enzymatic catalysis*[11]

	homogeneous catalysis	enzymatic catalysis
Enantiomers	both accessible	generally one enantiomer
Reaction diversity	large	small
Substrate scope	large	small
Solvent preference	mostly organic	mostly aqueous
Optimization	chemical	genetic
Second coordination sphere	less defined	more defined
Turnover numbers	smaller	larger

As summarized in Figure 5.2, although operating in apparently parallel universes, asymmetric homogeneous catalysis and biocatalysis have the preparation of

enantiomerically pure compounds as a common goal. On one side, new routes using metal-catalyzed approaches to target chiral molecules are continuously explored; on the other side, there is a continuous search for enzymes with novel catalytic properties. Similarly, once a hit has been found, improvements of the performance of the catalyst are achieved by its iterative redesign or modification.

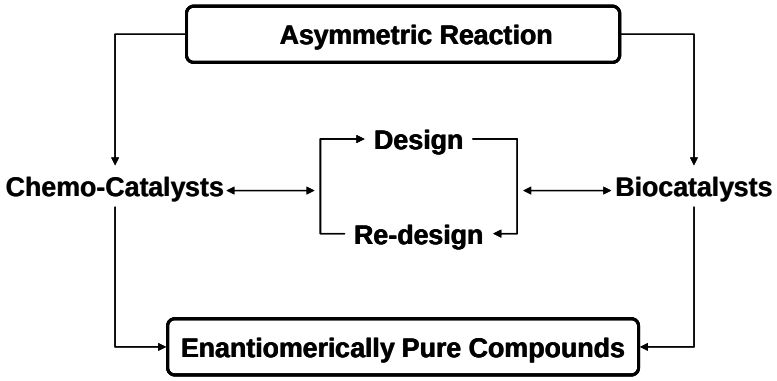


Figure 5.2 *Chemistry and biochemistry: different approaches, similar goal*

Moreover, the idea of cooperation (for example in cascade reactions) between the two fields starts to emerge as an appealing opportunity to expand the toolbox of modern organic chemistry and the implementation of catalysis in the preparation of chiral molecules.[12]

5.2 Scope of this study

An even more intriguing development, going beyond their simple cooperation, would be the fusion of these two approaches by the insertion of non-chiral metal catalysts into the active site of an enzyme. In this way, the enzyme will provide the chiral environment and the chemo-catalyst will allow the enzyme to perform new types of catalysis, thereby broadening the scope of both fields (Figure 5.3). Once the methodology has been established, this approach would also allow to take advantage of the tremendous advances in molecular biology techniques, such as gene manipulation and functional selection methodologies, for the preparation of large libraries of proteins, expanding even further the combinatorial approach for the identification of suitable catalysts for all kinds of transformations.[13,14]

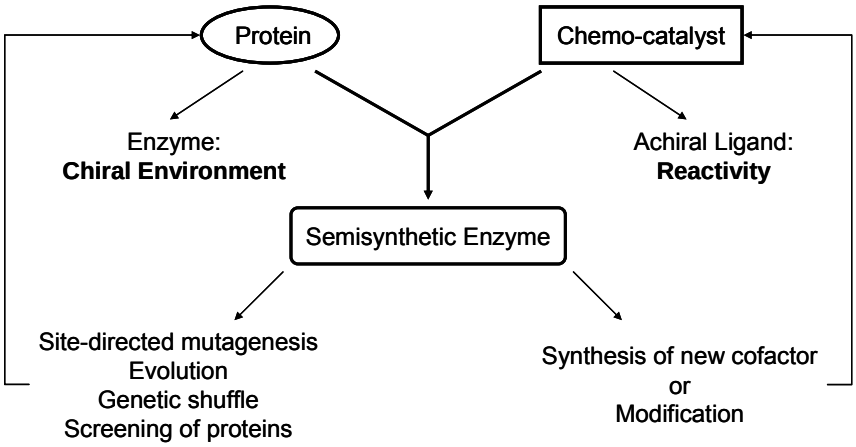


Figure 5.3 *Achiral ligands and proteins scaffolds as source of novel catalysts*

Based on the highly successful use of monodentate phosphorus ligands in asymmetric catalysis,[5] this study focused on the challenging goal of building a hybrid enzyme-bound rhodium catalyst with a single phosphorus donor ligand for hydrogenation and hydroformylation.

5.3 The pioneering work of Kaiser and Whitesides

The first examples of the preparation of semisynthetic enzymes appeared in 1966 by Bender[15] and Koshland[16] and their coworkers. It consisted in the chemical replacement of the serine residue with a cysteine in the active site of subtilisin. The enzyme lost its ability to hydrolyze amide bonds but maintained the esterase activity.

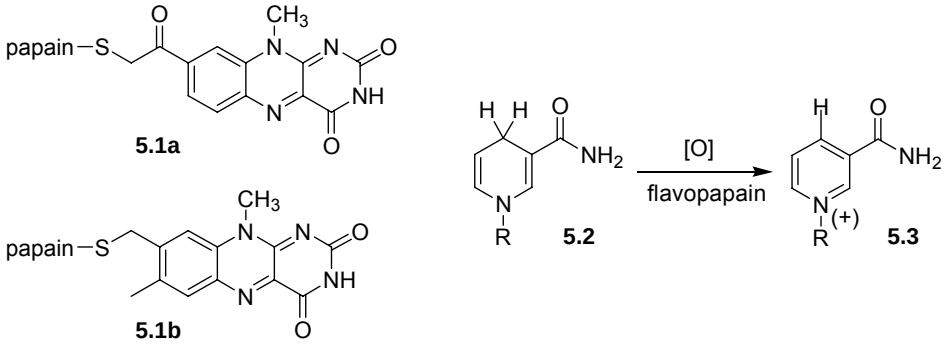
Chemical modification of residues in proteins was known before these studies and was used to investigate and identify important residues in the proteogenic structures. In this case, the fundamental difference was the intention to actually use the resulting semisynthetic enzymes for biocatalytic transformations. These early attempts not only established the synthetic methodologies, but most importantly demonstrated that, while working with enzymes, researchers could be not only spectators but also designers of enzymatic activities.

Twenty years later, Kaiser and coworkers demonstrated the usefulness of the modified thiol-subtilisin as peptide ligase.[17] The same concept was utilized also by Hilvert and Wu[18] for the preparation of a seleno-subtilisin with peroxidase activity.[19] There have been a number of examples in the literature, in which residues were changed in order to obtain different specificities.[20] During the years, the concept evolved from chemical modifications to even more sophisticated protein engineering such as introduction of non-natural amino acids, peptide ligation and genetic techniques. Nevertheless, these approaches limit the modifications to the

enzyme backbone and to the interconversion of reactivity or specificity among enzymes.

Another fundamental step towards a better understanding of the potential of protein structural manipulation is represented by the milestone work of Kaiser,[21] Whitesides[22] and their coworkers. They were the first, in the late seventies, to envision the possibility of inducing *new* catalytic properties by introducing artificial cofactors into protein scaffolds. Nevertheless, although motivated by the same pioneering spirit, they undertook two completely different approaches.

Kaiser and co-workers hypothesized that it was possible to use the cysteine residue in the active site of papain in order to chemically introduce a cofactor able to perform a reaction previously unknown to the enzyme.[21] Consequently, the irreversible modification resulted in the complete loss of the original proteolytic activity. Flavins were chosen as cofactors (Scheme 5.2), since they are quite effective oxidation catalysts even in the absence of the protein scaffold.

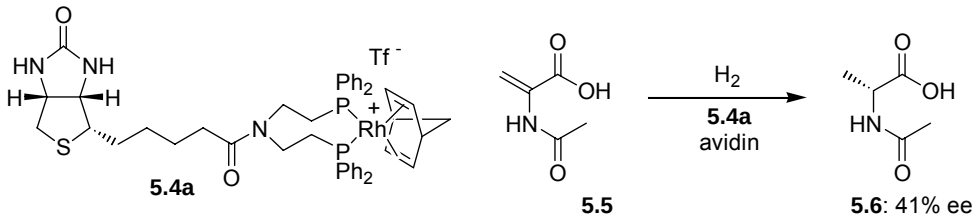


Scheme 5.2 *Flavopapains **5.1a-b**, as first example of covalently attached cofactors*

Therefore, it seemed likely that flavoenzymes could be generated without the involvement of specific α-amino acid functional groups in the protein for the new catalytic reaction to occur. As a result, papain modified with different flavins (**5.1a-b**) was indeed an active catalyst in the oxidation of NADH analogs **5.2**, reaching up to 670-fold rate acceleration compared to the corresponding flavin. Moreover, a preference for the abstraction of the *pro R* hydride of **5.2** was observed, which would suggest the involvement of the chiral environment of the protein scaffold.[23]

On the other hand, Whitesides and Wilson described an approach for the construction of an asymmetric hydrogenation catalyst based on the embedding of an achiral catalyst in a protein at a specific site.[22] The protein tertiary structure would provide the necessary chiral environment required for the enantioselective hydrogenation to occur. Again as a consequence, the protein would be equipped with new catalytic properties.

For this purpose, avidin was chosen because it was a well-characterized globular protein composed of 4 identical subunits. Most important, each subunit was known to bind a molecule of biotin so tightly that the association could be considered effectively irreversible.



Scheme 5.3 *Biotin-avidin technology used in asymmetric hydrogenation*

Therefore, as shown in Scheme 5.3, biotin was modified with an achiral Rh-diphosphine catalyst and the adduct **5.4a** was active in the hydrogenation of 2-(acetamido)acrylic acid (**5.5**). In the presence of avidin, *N*-acyl alanine (**5.6**) was obtained with 41% enantioselectivity.

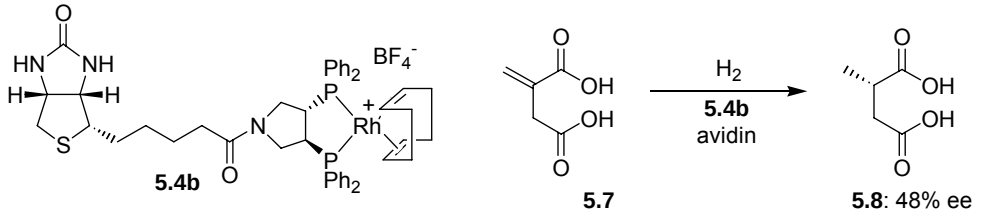
5.4 Artificial metalloproteins: state of the art

The concept of hybrid biocatalysts, as proposed by Kaiser and Whitesides, seemed to have been forgotten for almost twenty years. However, in the last decades, biotechnology, bioengineering and computational techniques evolved tremendously, together with the understanding of why certain protein structures exist and how they operate. Between the different protein families, metalloproteins have been receiving increasing attention as they are among the most efficient and diverse biocatalysts. Consequently, there is a growing interest in bio-inorganic chemistry and the boundary aspects of this kind of research.[24] The modification of the binding properties, metal centers and metal-containing cofactors seem to offer access to different complexes with potentially broad applications.[25] These applications include affinity purification of proteins, improved and metal-mediated protein stability, imaging and therapy, biosensors and possibly new catalysts.[26] Nevertheless, nature offers only a limited amount of ligands, metals and metal-containing prosthetic groups compared with what is provided by metal-coordination chemistry. Therefore, the introduction of *non-natural* metal containing cofactors seems to be a *natural* evolution toward novel biocatalysts.[27]

Technical advances and changes in the scientific community's perspective seem to be at the origin of a renewed interest in the construction of artificial metallo-enzymes.[28] The growing efforts towards their creation have been mainly concentrated on either covalent incorporation of cofactors or supramolecular approaches.[29]

5.4.1 The non-covalent approach

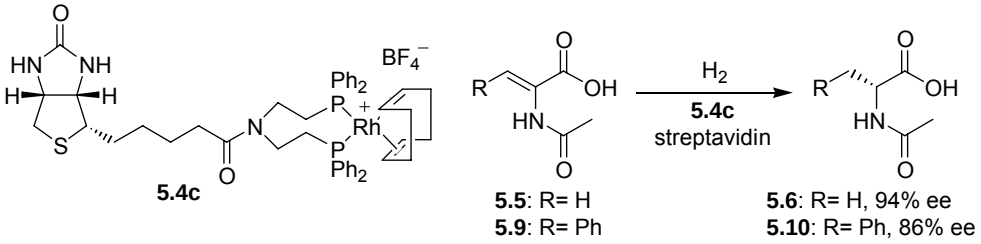
Chan and coworkers were the first to reconsider the work of Whitesides. In their extension of this approach, biotin was modified with a chiral bidentate phosphorus ligand **5.4b** and used in the hydrogenation of itaconic acid (**5.7**).[30]



Scheme 5.4 *Extension of Whitesides approach using a chiral diphosphine based cofactor **5.4b** in the hydrogenation of itaconic acid (**5.7**)*

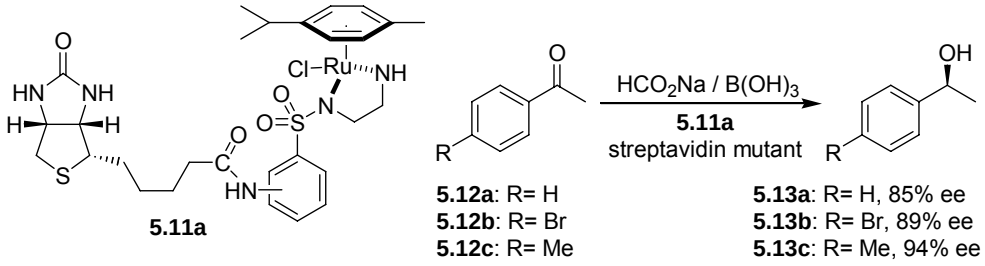
Using the cofactor **5.4b** shown in Scheme 5.4 product **5.8** was obtained with 48% ee, whilst using the cofactor containing the ligand with opposite configuration only 16% ee was found. The results, even if not exciting, demonstrated the influence of the tertiary structure within the protein cavity.

More recently, Ward and coworkers[31] obtained remarkable results (Scheme 5.5) optimizing the approach of Whitesides by using streptavidin, another biotin-binding protein which displays similar affinity but only 35% sequence homology. The main difference appeared to be the presence in streptavidin of a deeper binding pocket. Furthermore, site specific mutagenesis allowed to obtain *N*-acyl alanine (**5.6**) with an increased 96% ee.



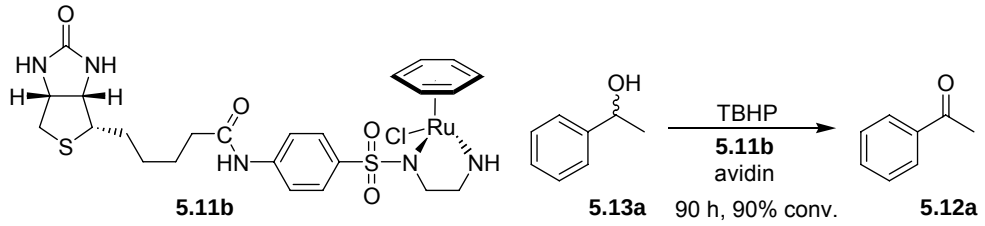
Scheme 5.5 *Optimization of Whitesides approach using a streptavidin adduct of **5.4c** and site specific mutagenesis*

The authors extended the approach to transfer hydrogenation of methyl aryl ketones (**5.12a-c**).[32] The new cofactor **5.11a** was prepared by modification of biotin with an analog of Noyori's amino sulfonamide scaffold complexed with $[\eta^6$-(p-cymene)$RuCl_2]_2$ (Scheme 5.6). In the presence of mutants of streptavidin, the hybrid catalyst was able to induce up to 94% ee in the hydrogenation of *p*-methyl acetophenone **5.12c** using formate-boronic acid as reducing agent.



Scheme 5.6 *Artificial metallo-enzyme as catalyst in transfer hydrogenation using biotin-streptavidin technology*

Using the same biotin-(strept)avidin technology, Ward and coworkers investigated also the reverse reaction, namely the oxidation of alcohols (Scheme 5.7).[33] In this case, avidin resulted to be the best host for biotin modified with the amino-sulfonamide Ru(II) catalyst **5.11b**. The hybrid metalloprotein was able to convert phenethyl alcohol (**5.13a**) into the corresponding acetophenone (**5.12a**) in the presence of TBHP as oxidizing agent.



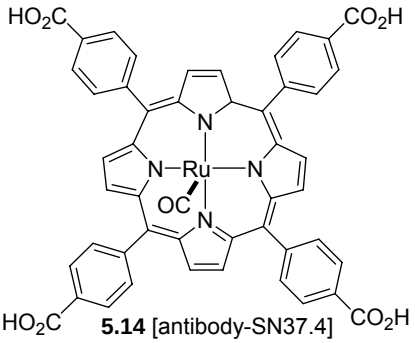
Scheme 5.7 *Artificial metallo-enzyme using biotin-avidin technology as catalyst for alcohols oxidation*

Keinan and Nimri[34] were the first to propose a metalloporphyrin-antibody (SN37.4) assembly **5.14** and to adopt it as model of a heme-dependent enzyme. Using Ru(II) as metal center, 43% ee was obtained in the oxidation of thioanisole (**5.18a**) using PhIO as oxidizing agent (Scheme 5.8).

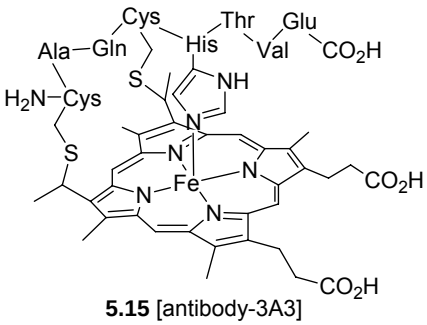
More recently, Mahy and coworkers also reported an antibody (3A3) containing a Fe(II)porphyrin complex **5.15**.[35] In this case, trying to overcome the intrinsic lower reactivity associated with artificial hemoproteins, they decided to equip the phorphyrin moiety with a heme octapeptide (MP8) containing a histidine able to act as extra ligand for the metal center. The artificial metallo protein was tested in the oxidation of thioanisole (**5.18a**) and 45% ee was obtained using H_2O_2 as oxidizing agent. Nevertheless, the activity of this system was still not comparable with the activity of authentic hemoproteins.

In their investigation, Watanabe and coworkers developed mutants of apo-myoglobin (apo-Mb) reconstituted with Cr(III) Schiff base complex **5.16**, known to be able to perform oxidation reactions.[36] Apo-myoglobin was chosen as its

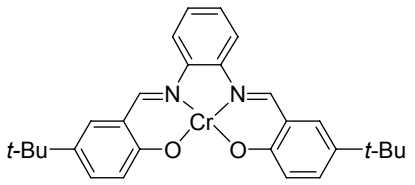
reconstitution with heme is well documented. For example, as it is well known that the binding affinity of heme to apo-Mb is caused by hydrophobic interactions, they introduced two *t*-butyl groups in the 5-, 5'- positions of salophen. The artificial metallo-enzyme obtained was tested in the H_2O_2-dependent sulfoxidation of thioanisole (**5.18a**) providing 13% ee.



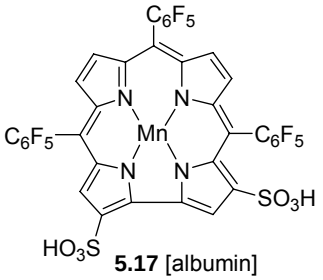
5.14 [antibody-SN37.4]



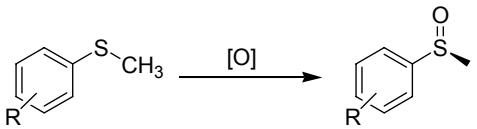
5.15 [antibody-3A3]



5.16 [apo-myoglobin]



5.17 [albumin]



5.18a: R= H
5.18b: R= 2-Br

5.19a: R= H
5.19b: R= 2-Br

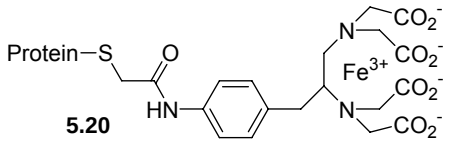
5.14: **5.19a**, 43% ee (S)
5.15: **5.19a**, 45% ee (R)
5.16: **5.19a**, 13% ee (S)
5.17: **5.19b**, 74% ee (S)

Scheme 5.8 *Use of artificial metallo-enzymes in the sulfoxidation of thioanisole*

The most recent artificial hemoprotein was presented by Gross and Mahammed.[37] They adopted Fe(III) and Mn(III) amphiphilic corrole complexes **5.17** conjugated with albumin (HSA). Mn-corroles have been studied in recent years by the authors for their potential in oxidation catalysis; moreover the amphiphilic sulfonated version **5.17**, depicted in Scheme 5.8, was shown to form stable conjugates with HSA, which is a cheap and readily accessible protein.[38] Once more, the biomimetic system was tested in the oxidation of a number of aryl methyl sulfides **5.18** reaching up to 74% ee (**5.19b**) with various degrees of activity.

5.4.2 The covalent approach

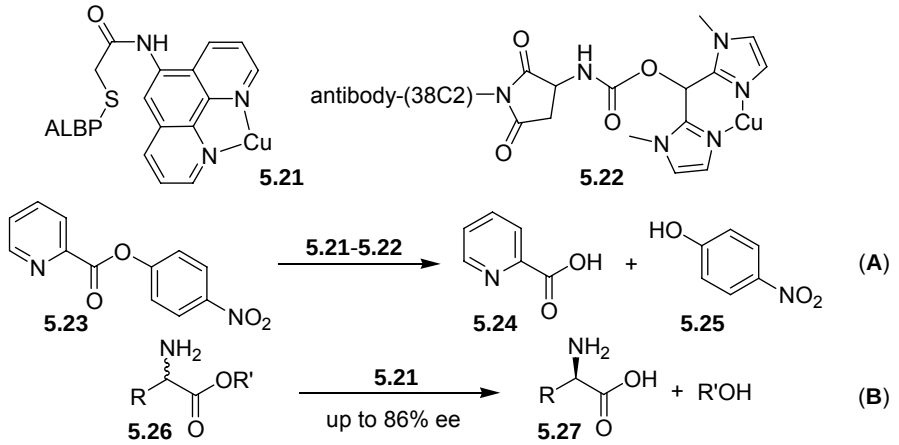
In the covalent approach, following the pioneering study of Kaiser, a number of examples of chemical modification of reactive residues in proteins appeared in the literature.[20] In the field of artificial metalloproteins, Meares and Rana[39] attached a Fe-EDTA chelate (Fe-BABE) to Cys-212 of human carbonic anhydrase I (HCAI), the adduct **5.20** was able in the presence of ascorbate and H_2O_2 to cleave selectively HCAI itself after Leu-189 in the peptide chain (Scheme 5.9). The hydrolysis was sensitive to the proximity of the attached reagent more than the presence of specific residues. Moreover, Meares and coworkers applied this approach to *E. coli* RNA polymerase, a DNA-binding protein, which was used in affinity-cleavage experiments in order to identify interaction-sites between nucleic acids and RNA polymerase.[40]



5.20

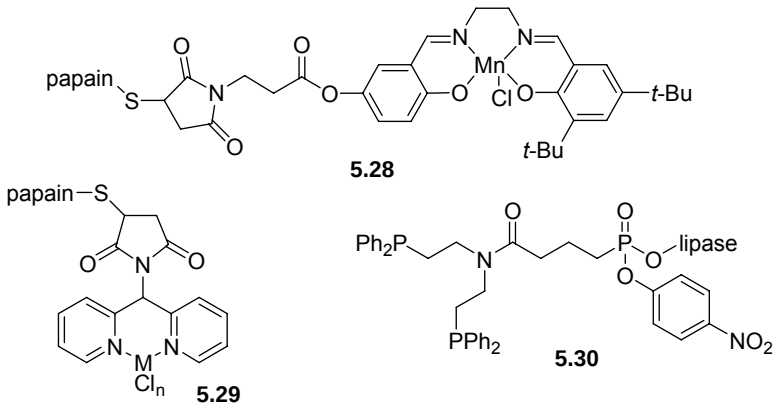
Scheme 5.9 *Artificial iron-dependent protease **5.20***

Davies and Distefano,[41] introduced a Cu(II) 1,10-phenantroline complex **5.21** in adipocyte lipid binding protein (ALBP); whilst Janda and coworkers[42] proposed an aldolase antibody (38C2) derivatized with a Cu-binding bis-imidazolyl cofactor **5.22**; both systems showed hydrolytic activity in the presence of activated esters (**A**, Scheme 5.10) such as picolinic acid nitrophenyl ester (**5.23**). Additionally, the system **5.21** adopted by Davies and Distefano showed also to be active in the presence of simple α-amino acids alkyl esters **5.26** (**B**) providing enantioselectivities between 31% and 86% for the α-amino acids **5.27** obtained.



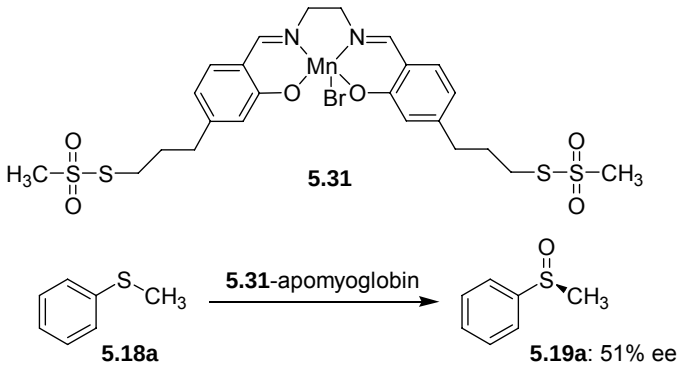
Scheme 5.10 *Cu-based artificial metalloproteins **5.21-5.22** with hydrolase activity*

Moving away from these interesting but still more traditional examples of hybrid metallo-enzymes, Reetz and coworkers reported the introduction in papain of maleimide based manganese-salen **5.28** and Rh-dipyridin **5.29** complexes as epoxidation and hydrogenation catalysts, exploiting the maleimide unit as Michael acceptor for the reactive Cys-25 (Scheme 5.11).[43] Although no clear experimental documentation was provided, the new biocatalysts are reported to be able to promote hydrogenation and epoxidation reactions, even if the enantioselectivities obtained were less than 10%. They also proposed a diphosphine-based phosphonate inhibitor **5.30** (Scheme 5.11), known to react with the catalytically active serine lipases resulting in covalent inhibition of the residue. Unfortunately, the activity was largely restored within a day, due to the hydrolysis of the phosphonate moiety from the lipase, not surprisingly as such a phenomenon is known[44] and in this case was accentuated by the presence of a labile *p*-nitro-phenol substituent on the phosphonate.



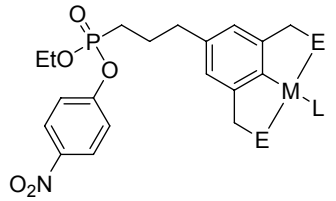
Scheme 5.11 *Hybrids biocatalysts **5.28-5.30** envisioned by Reetz and coworkers*

Another example of covalent cofactor attachment, in this case of a manganese-salen complex **5.31** into apo sperm whale myoglobin (Apo-Mb), has been recently presented by Lu and coworkers and tested in enantioselective sulfoxidation reactions (Scheme 5.12).[45] The cofactor introduced is similar to the one proposed by Watanabe (**5.16**, Scheme 5.8) following a supramolecular approach and by Reetz (**5.28**, Scheme 5.11) using a covalent approach. The special feature of this work consisted in a double mutation that allowed anchoring the cofactor to two cysteine residues, limiting the conformational freedom. The approach resulted to be successful as 51% ee was obtained in the oxidation of thioanisole (**5.18a**), compared to 12% ee provided by the same cofactor **5.31** but with only one point attachment. Moreover, the double anchoring of the cofactor proved also to be beneficial for the reactivity.



Scheme 5.12 *Two points attachment of a Mn-salen complex **5.31** to Apo-Mb*

Recently, van Koten and coworkers[46] reported the modification of cutinase (a lipase) with "pincer"-type metal complexes **5.32a-c** using an approach similar to the one reported by Reetz (**5.30**, Scheme 5.11).[43b] The phosphonate adducts described in this work were found to be stable and no dissociation was observed (Scheme 5.13). Nevertheless, these modified proteins have not been reported to possess any kind of catalytic activity.



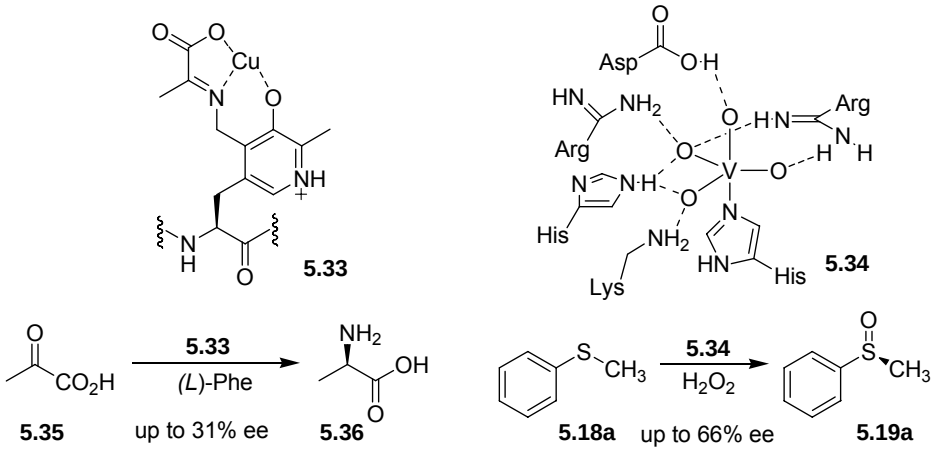
5.32a: E= NMe_2, M= Pt, L= Cl
5.32b: E= SPh, M= Pd, L= Br
5.32c: E= SMe, M= Pd, L= Br

Scheme 5.13 *"Pincer"-type metal complexes **5.32a-c** used in the modification of cutinase*

5.4.3 Other approaches for the incorporation of metal centers

Beside the two main lines of research presented, there have been reports of different approaches for the incorporation of metal centers in proteins.[47] For example, using solid phase methodology, Imperiali and Roy prepared a semisynthetic RNase-S protein incorporating an unnatural amino acid with a pyridoxamine moiety **5.33** which displayed transaminase activity (Scheme 5.14).[48] Suckling and coworkers replaced existing naturally occurring zinc in carboxypeptidase A (CPA) with different metals.[49] The exchange of Zn with Ni, Co, Rh proved successful, whilst denaturation of the protein occurred with Ru and Pd. The metal-CPA adducts prepared were used in hydrogenation reactions, nevertheless, no activity was observed. Sheldon and coworkers, instead, used different phytases in order to introduce transition-metal oxoanions such as VO_4^{3-}, taking advantage of their known inhibitory power toward these proteins (Scheme 5.14).[50] The semi-synthetic vanadium peroxidase **5.34** obtained showed to be

active, in the presence of H_2O_2, for the oxidation of prochiral sulfides **5.18** reaching up to 66% ee. Marchetti and coworkers used non-specific binding of $Rh(acac)(CO)_2$ on HSA in order to successfully perform hydroformylation reactions in a two phases system.[51,155] The use of different proteins such as papain and egg albumin was found to be less efficient.[52]



Scheme 5.14 *Alternative approaches for metal-incorporation into proteins*

Finally, theoretical approaches have been developed using automated computer search algorithms, such as Metal-Search[53] and Dezymer,[54] in order to design novel metal-binding sites into proteins.[25]

5.5 The importance of the appropriate strategy

At the beginning of this endeavor, the main reference points, among those previously described, were still the studies of Kaiser[21] and Whitesides.[22] The analysis of their approaches helped clarifying the decisions that needed to be made as depicted in Figure 5.4.

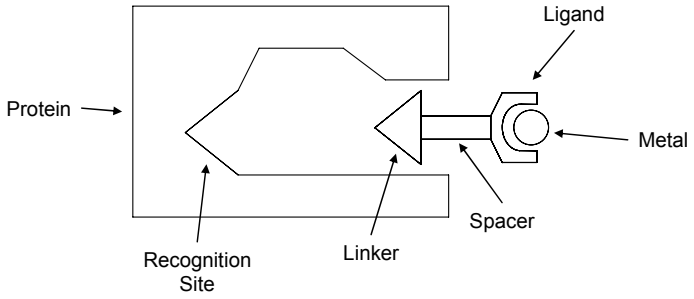


Figure 5.4 *The various aspects of the strategy require several choices*

The objective is to equip a protein with an artificial coenzyme[55] that would perform metal-catalyzed transformations unknown to the native protein itself. With this aim in mind, it is necessary to identify suitable proteins with the following properties:

- Good availability and accessible biochemical knowledge.
- Specific (1:1 ratio) and stable adducts between the protein and the cofactor.
- Generality of the approach that could be extended to different proteins as well as different cofactors.
- Minimum destabilization of the protein itself due to the interactions with the cofactor.

5.5.1 Protein classes

Proteins can be divided into two main groups: fibrous and globular. Fibrous proteins play a structural function and are characterized by their insolubility which makes them not suitable for this study. Globular proteins are instead globelike proteins more or less soluble in aqueous solution and can be divided according to their functions into messengers, transporters (binding proteins) and enzymes (Figure 5.5).[56] Transport proteins and enzymes are a desirable choice due to their availability and the specificity of either their binding properties or reactivity.

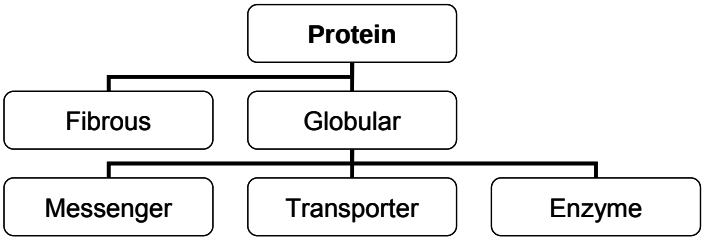


Figure 5.5 *Protein classification*

Among the different transporters of major interest are those proteins with affinity for organic molecules. For example, proteins such as hemoglobin and myoglobin contain a heme unit which is responsible for the actual transport of oxygen. Therefore, the specificity and stability of the binding of the ligand is extremely important and effective. The serum protein albumin is known for being responsible for the transport of a variety among metals and more interestingly lipophilic molecules. Avidin and streptavidin are well known for their high affinity for biotin and are already widely used in immunoassays and affinity chromatography. The replacement or modification of these tightly bound molecules with others with the same binding capabilities, but designed to have interesting catalytic properties, is at the origin of the non-covalent approach of which the work of Whitesides[22] is an excellent example. A desirable feature of the supramolecular approach is the limited amount of protein manipulation required.

On the other hand, enzymes are defined as proteins that catalyze and accelerate a variety of chemical reactions by efficiently bringing substrates together in a so called enzyme-substrate complex located in a specific region defined as “active site”.[57] The active site of an enzyme also contains the residues (catalytic groups) directly involved in the making or breaking of bonds and possess higher reactivity than any other identical residue present elsewhere in the protein. This specific reactivity in a well-defined position of the enzyme makes these catalytic residues very attractive and practical tools for chemical mutation, which is at the origin of the covalent approach adopted by Kaiser.

The use of enzymes as hosts for artificial cofactors guarantees the availability of an enormous reservoir of potential structures, making them a desirable choice for the establishment of a general method. The success of the introduction of a new cofactor by chemical alteration relies mainly on the reactivity of the residue itself and not on delicate and fundamental non-covalent interactions involved in a strong and selective binding. Moreover, the number of proteins which display this kind of binding for non proteinogenic molecules is limited. For example, the use of proteins such as avidin or myoglobin would restrict the design to cofactors preserving the natural binding capabilities of biotin and heme respectively.

In consideration of all these factors, it was decided to follow a covalent approach and introduce the artificial coenzyme by selective chemical modification of a specific residue in the active site of a given enzyme.

5.5.2 Enzyme families

Enzymes are generally classified according to the kind of reaction performed and the different categories are depicted in Figure 5.6.[58]

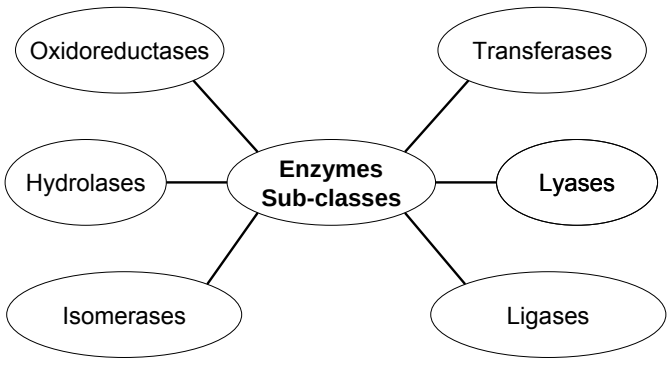


Figure 5.6 *Enzymes classification*

Among the different families of enzymes the hydrolases are of particular interest. Their mechanism of action is very similar to a base-catalyzed chemical hydrolysis. Stability, lack of sensitive cofactors and wide substrate tolerance makes them very popular also in organic chemistry applications.[8] Hydrolases are further divided in

thirteen sub-classes according to their reactivity toward specific bonds and the most common are esterases, glycosylases and peptidases.

Peptidases (or proteases) are the enzymes involved in the hydrolysis of amide (peptide) bonds and they are the most abundant and studied among the hydrolases family as they play a role in a variety of physiological processes.[59] Furthermore, they are commonly used in sequence analysis and domain identification of other proteins. The proteolytic enzymes are further classified, according to the catalytically active residues, mechanism of action and three-dimensional structure. Each family possesses a particular set of residues which arranges similarly to form the active site. The most representative enzymes and their characteristic active site residues are depicted in Figure 5.7.[60] These four families are grouped according to their mechanism of action: serine and cysteine proteases, which form covalent enzyme complexes with the substrate; aspartic and metallo-proteases, which do not, making them less interesting in this context.

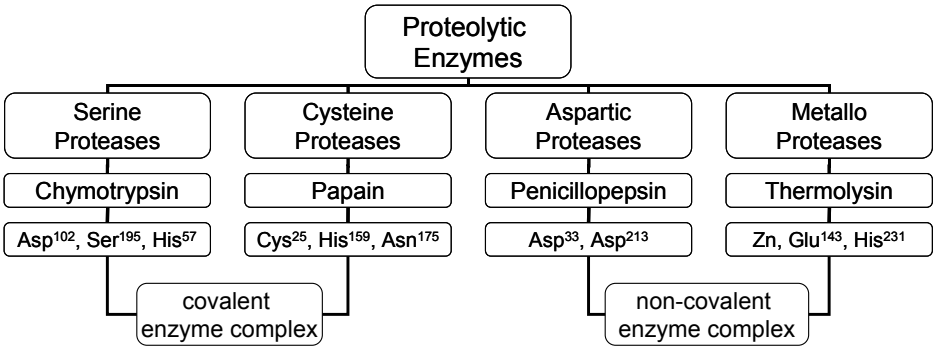
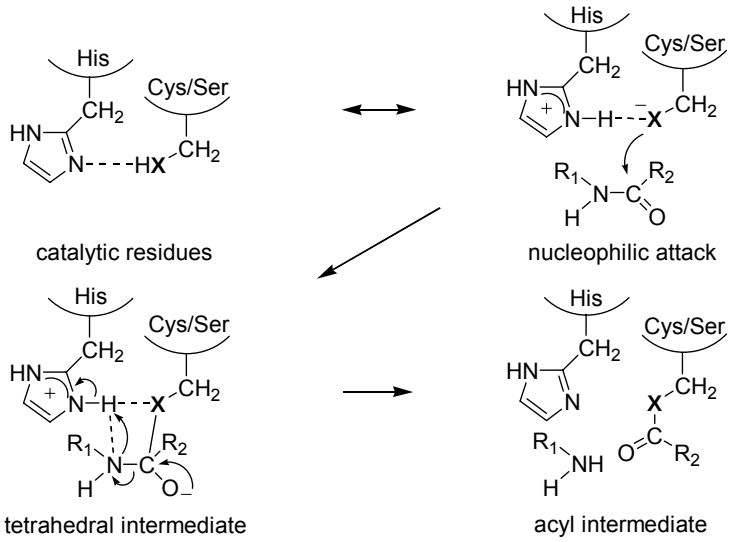


Figure 5.7 *Proteolytic enzymes divided according to the residues in the active site*

Serine and cysteine proteases, which are small monomeric enzymes of M_r between 15,000 and 35,000,[61] are the most well known among the proteolytic enzymes and they share similar mechanism of action and basic characteristics.[62] The involvement of serine proteases in digestion processes made them the object of early extensive studies that provided the basics for most of the current knowledge on protein structure and enzyme function. Investigations on the kinetics, specificity of inhibition, analyses of the α-amino acid sequence, X-ray structure and site direct mutation led to the identification of the essential residues and mechanisms of action.

The extensive theoretical and practical knowledge available about these enzymes makes them good candidates for the introduction of an artificial cofactor using a covalent approach. These proteolytic enzymes have strongly nucleophilic serine or cysteine residues in the active site, whose reactivity is enhanced by the presence of a spatially aligned histidine residue (Scheme 5.15).



Scheme 5.15 *Similar mechanism of action of serine and cysteine proteases*

Modification of the active residues serine and cysteine by acylation or alkylation, taking advantage of their intrinsic reactivity, has been extensively used as powerful tool to study the mechanism of action, spatial requirement and possible intermediates during catalysis.[63] Therefore, the use of the available knowledge in enzyme inhibition seems to be the most straightforward and reliable way of chemically introducing an organic molecule in a specific position of an enzyme and will be discussed more in detail in a separate section (page 188).

The specificity of the reactivity allows control over the position in which the cofactor is positioned and over the number of cofactors introduced. In this respect cysteine proteases start to emerge as more desirable, due to the limited amount of cysteine residues present in enzymes and proteins, which are generally involved in disulfide bonds of structural importance; so if a single free cysteine is present it becomes a perfect target. With a broader perspective, the higher nucleophilicity of cysteine side chains (pK_a 8.5-9.5) compared to serine ones (pK_a 13), together with their limited presence, allows one to envision their potential artificial introduction in any desired position of generic protein structures maintaining the specificity of the anchoring of the cofactor.[64]

5.6 Cysteine proteases: papain

Cysteine proteases, that are referred to as thiol proteases in the old literature, are enzymes widely distributed in nature and have been found in viruses, bacteria, protozoa, plants, and more recently in fungi.[65] Among the many cysteine proteases isolated from plants the most commonly known are papain (from papaya), ficin (from figs), bromelain (from pineapple), and actinidin (from kiwi fruit) and they are all members of a structurally homologous family.[66]

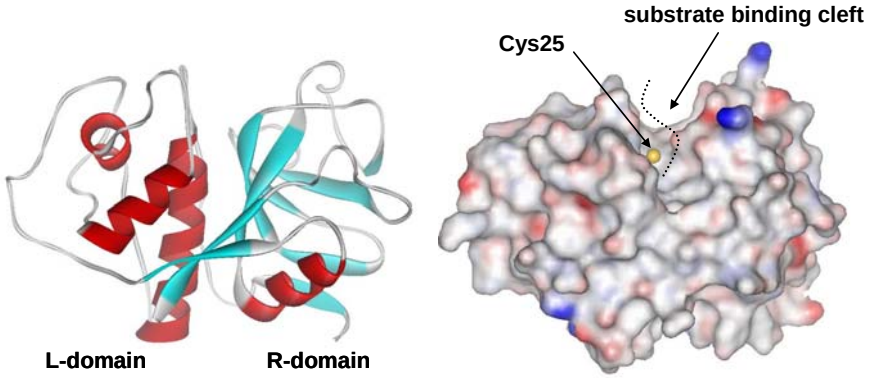


Figure 5.8 *Three-dimensional structure of papain with domains and binding cleft*

The best understood enzyme in this family is papain (EC 3.4.22.2),[67] which is a small and monomeric protein of 212 residues and molecular weight of 23428 Da.[68] Papain has been extensively studied and it is considered the main representative of its family,[69] also because it was the first cysteine protease structure solved at high resolution (2.8 Å) by Drenth and coworkers and later refined by Kamphuis and coworkers (1.65 Å).[70] The analysis of the x-ray structure shows that the polypeptide is folded into two distinct parts, L and R domains, which are divided by a cleft of about 15 Å with a groove of about 25 Å (Figure 5.8).[71] The groove was found to be able to accommodate peptides constituted of up to seven amino acids residues (P, P') binding to an equal number of subsites (S, S') as depicted in Figure 5.9.[72] The catalytically essential residues His-159 and Cys-25 can also be found in the groove, located on the opposite domains of the cleft. Besides four short α-helical segments and one short segment of β-structure, the conformation of the chain is irregular. An interesting feature of the secondary structure of papain is the presence of 7 cysteine residues, of which 6 are involved in structurally relevant disulfide bonds, making Cys-25 not only the most reactive but also the only available catalytically-active cysteine in the enzyme.[69]

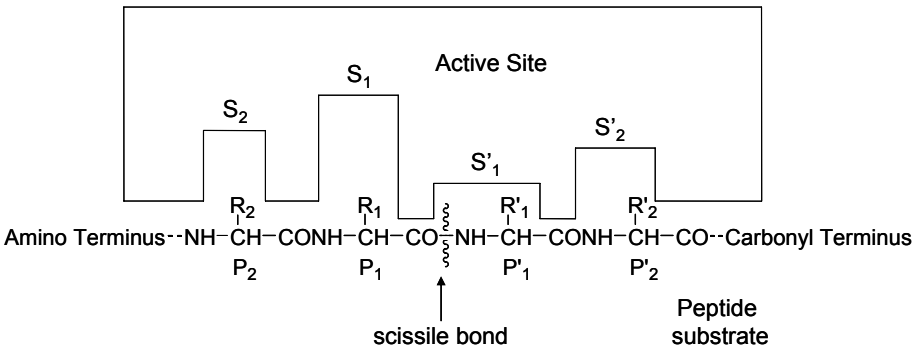
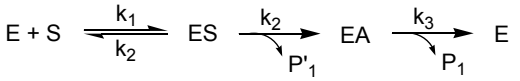


Figure 5.9 *Subsites S, S' and residues P, P' distributing on the sides of the scissile bond*[73]

Peptides and esters are hydrolyzed through an acyl-enzyme pathway in the same manner serine proteases are, except that in this case Cys-25 is acylated (Scheme 5.15) and the rate determining step in the hydrolysis of amides and anilides appears to be the general-acid-catalyzed breakdown of this intermediate (Scheme 5.16).



E= free enzyme
S= substrate
ES= enzyme-substrate complex (or Michaelis complex)
EA= acyl enzyme
P'_1= amine product
P_1= acyl product

Scheme 5.16 *Intermediates in papain catalysis*

Numerous studies have been conducted on the reaction mechanism of papain and the role of some of the residues.[66] These investigations generated a huge amount of knowledge on this enzyme and other cysteine proteases, although there is still disagreement on many details. Generally speaking, the high nucleophilicity of papain is caused by the thiolate-imidazolium ion pair formed by the interaction of the dissociated Cys-25 (pK_a 8.5) and the protonated His-159 (pK_a 4).[74] This interaction is also suggested by the relative position of these two residues (3.4 Å) in x-ray diffraction studies.[75] The low pK_a of the imidazole group of His-159 is attributed not to the interaction with an aspartic acid as in serine proteases, due to the unfavorable position, but more to the interaction with the side chain of Asn-175 (Figure 5.10).[76,77] More recently, Ménard and coworkers further confirmed the role of Asn-175 by investigating the reactivity of papain in which the residue was substituted for other residues using site-directed mutagenesis.[78]

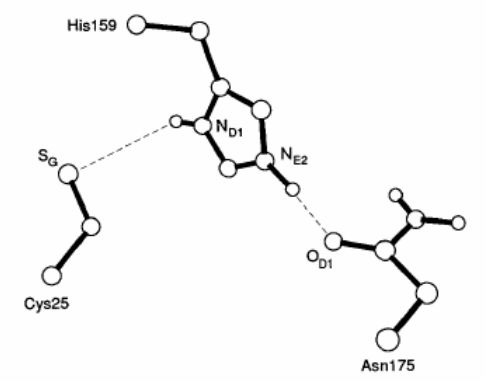


Figure 5.10 *Schematic representation of the active site showing the catalytic triad Cys-His-Asn*[79]

Unlike serine proteases, papain has a broad specificity and preference for hydrophobic α-amino acids. A deep non-polar secondary binding pocket, constituting hydrophobic side chains from Tyr-67, Pro-68 and Trp-69 on one side and those from Phe-207, Ala-160, Val-133 and Val-157 on the other, seems to be responsible for the preferential reactivity toward peptides with hydrophobic α-amino acids such as phenylalanine, tyrosine and leucine in P_2 (Figure 5.11).[80]

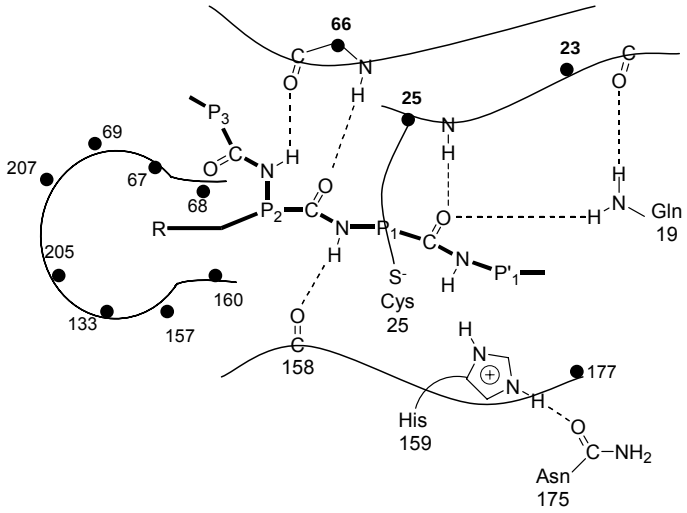
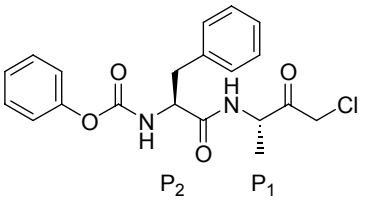


Figure 5.11 *Schematic representation of papain-substrate interactions*[81]

The presence of this pocket and steric interference with the bulk of the enzyme are also responsible for stereospecificity observed for L-amino acids. It was experimentally observed that the presence of phenylalanine in the peptide forces

the cleft to open somewhat and increase the strain of the active site.[82] A similar conclusion was reached analyzing the crystal structure of papain inhibited by the chloro-methyl ketone derivative of *N*-benzyloxycarbonyl-L-phenylalanine-L-alanine (**5.37**, Figure 5.12) where the estimation of the widening due to the presence of phenylalanine amounted to about 1 Å.[80] In this way, as shown in Figure 5.11, the side chain of phenylalanine would be able to nicely fit in the hydrophobic pocket, which confers extra stabilization to the binding of the substrate and/or to the acyl adduct. Beside this preference for an aromatic residue in P_2, papain possesses a rather broad specificity for what the other subsites (S) are concerned.



5.37: ZPACK

Figure 5.12 *Structure of ZPACK (**5.37**) used in x-ray diffraction as transition state analogue*

5.7 Low molecular weight synthetic inhibitors

The use of low molecular weight synthetic inhibitors has had a major impact on the knowledge achieved on the structure, reactivity and specificity of papain and enzymes in general.[73] A valuable example is the just mentioned use of ZPACK (Figure 5.12). Besides being used in exploratory research, inhibitors occupy an important position also in drug discovery as means to understand or interfere with the biological pathway of enzymes.[83,84] Therefore, there is a huge literature produced on the subject but also controversy on the definitions, which sometimes complicates the assimilation of the information. The following short overview of enzyme inhibitors and their classifications, limiting the examples to cysteine proteases inhibitors, helped identifying the most suitable way of introducing the cofactor into the active site of papain in a convenient, covalent and irreversible manner.

In general,[59] any compound that decreases the measured rate of hydrolysis of a given substrate is, in principle, an enzyme inhibitor which can be chemically reactive, catalytically processed or simply bound to the target enzyme.[85] As shown in Figure 5.13, a first classification divides inhibitors into active site directed inhibitors and allosteric effectors, compounds which interact temporarily with peripheral areas of the enzyme and those are not relevant in this study.

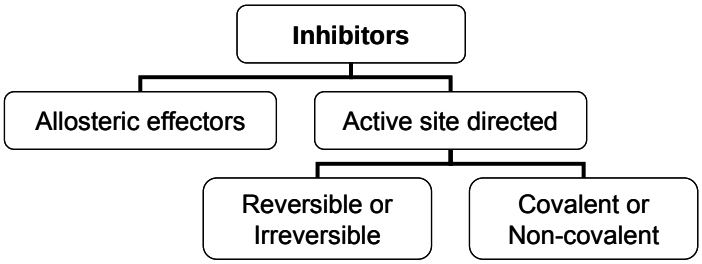


Figure 5.13 *Classification of inhibitors according to the nature of their interactions*

The inhibitors which target the active site can be classified according to their interactions into covalent or non-covalent and reversible or irreversible.[65] In the case of reversible inhibition, which usually involves a non-covalent interaction based for example on tight binding, the activity of the enzyme can be regained upon dilution or dialysis. Even if in some cases dissociation is so slow and the binding so strong that it could to be considered 'pseudo-irreversible', the non-covalent nature of the interaction makes this class not a desirable choice.

Covalent inhibitors interact with the enzyme *via* a chemical modification of the active site. These inhibitors are 'mechanism based' if they react with the active site following a normal catalytic process.[86] Otherwise, they are defined as 'affinity labels' if another chemical pathway, which has no connection with the catalytic process, is used (Figure 5.14).

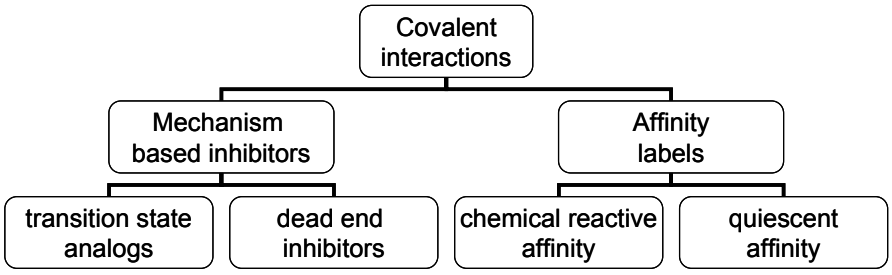
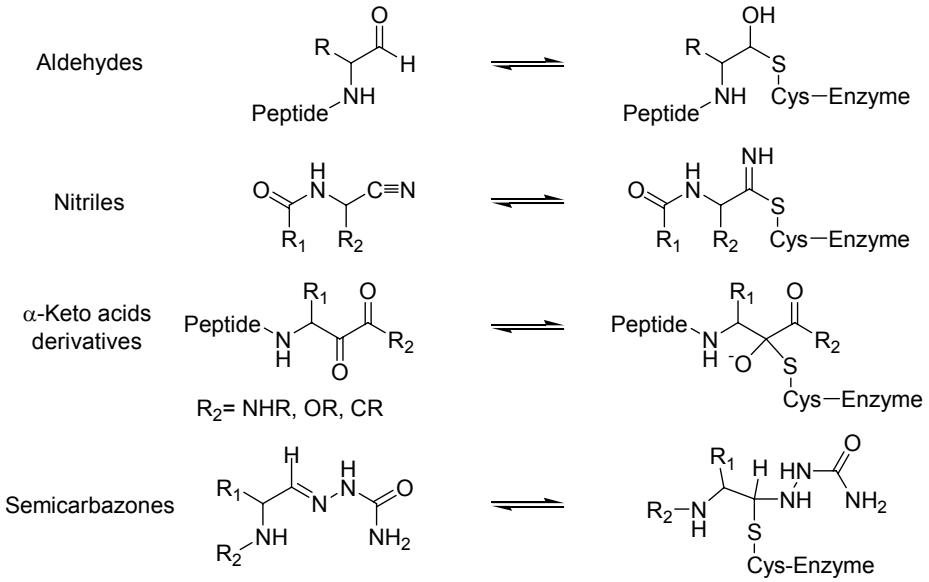


Figure 5.14 *Classification of covalent inhibitors according to the reaction mode*

Mechanism-based inhibitors are also defined as 'suicide substrates'. This means that the reactive functional group is *latent* in the molecule until the enzyme itself upon binding catalyzes its own destruction. As shown in Figure 5.14, these compounds that are converted into inhibitors during catalysis can be classified according to the product obtained after reaction with the enzyme into: transition state analogues when a tetrahedral adduct is formed but does not react further

(e.g. hemithioacetal with peptidyl aldehydes);[87] dead end inhibitors when a covalent complex which cannot react further is formed (e.g. thioimidate with peptidyl nitriles).[88]

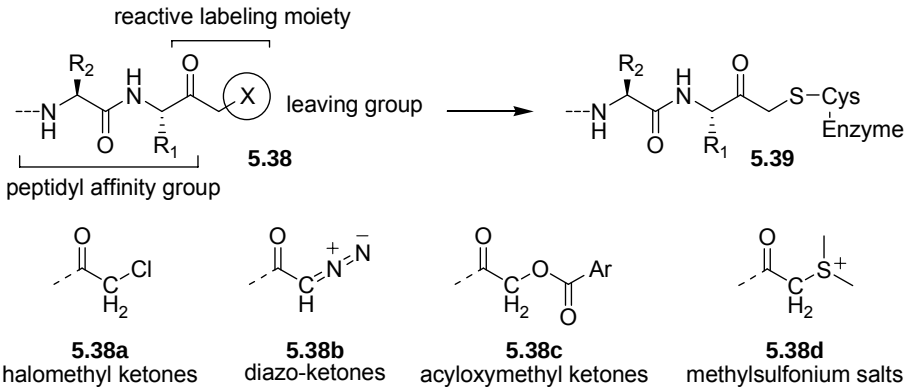
Unfortunately, covalent modification of the active site gives no guarantee of irreversibility in all cases. Reversibility can be encountered also when using covalently bound compounds because the bond hydrolyzes over time. Few examples can be found among the mechanism based inhibitors, such as peptidyl aldehydes or nitriles, but also α-keto acids derivatives and semicarbazones (Scheme 5.17).[65] The instability of these adducts, even if covalently bound and frequently used, makes them not suitable.



Scheme 5.17 *Covalently bound but reversible inhibitors frequently used*

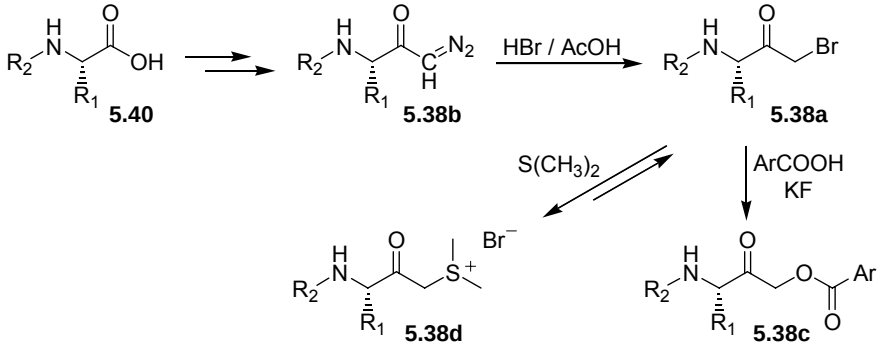
Affinity label inhibitors (Figure 5.14), which in some literature are defined as active site directed inhibitors,[86] are compounds possessing a functionality that reacts with the active site and, upon releasing a leaving group, form adducts which cannot be separated by gel-filtration or dialysis and cannot be hydrolyzed. The reaction pathway is not specific and does not recall the catalytic mechanism. Chemically reactive affinity labels are compounds that would be able to react with other molecules possessing the same reactivity of active site residues (e.g. halo ketones **5.38a**);[89] if instead the reagents have no reactivity for non-enzymatic molecules they are called quiescent affinity labels (e.g. acyloxymethyl ketones **5.38c**).[90]

Affinity labels **5.38**, as many other inhibitors, are characterized by a short peptidyl sequence used for binding recognition but they are divided according to the reactive leaving group present as depicted in Scheme 5.18.



Scheme 5.18 *Generic structure of peptidyl affinity label inhibitors **5.38** and most frequently used reactive leaving groups **5.38a-c***

Affinity label inhibitors **5.38** seem to have all the required characteristics. They are reactive compounds and the products of the alkylation reaction are truly irreversible. Halomethyl ketones **5.38a** are historically among the first affinity labels developed as a consequence of the high reactivity toward serine and cysteine proteases showed at first by small and simple reagents such as iodo acetic acid and iodo acetamide.[91] Many halomethyl ketones with different peptidyl sequences have been prepared as they played an essential role in the understanding of enzymes characteristics and inhibitors such as TPCK[92] (chymotrypsin) and ZPACK (papain, Figure 5.12).[93] The high reactivity and lower specificity of halomethyl ketones, especially for *in vivo* applications, stimulated the development of the other affinity labels depicted in Scheme 5.18, which were originally intermediates in the synthesis of the halomethyl ketones themselves or are their derivatives (Scheme 5.19).[90,94]

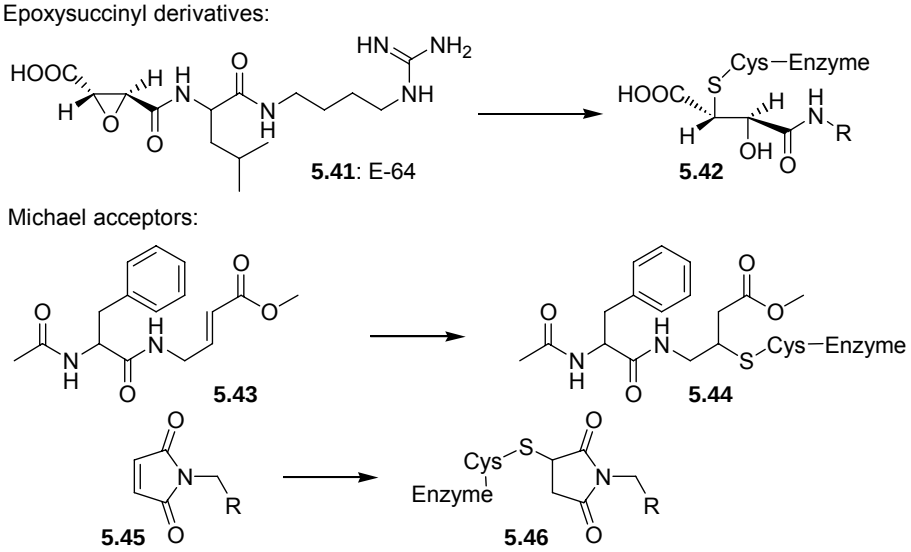


Scheme 5.19 *Chemical connection between the different inhibitors **5.38***

Diazoketone-based inhibitors **5.38b** are selective for cysteine proteases which makes them useful for *in vivo* inhibition, however, they are more effective at weakly

acidic pH, since protonation is required for the formation of the unstable diazonium ion. Methylsulfonium salts **5.38d**, besides having intermediate reactivity between halomethyl ketones **5.38a** and diazo-ketone reagents **5.38b**, have an intrinsic instability which might result in an internal displacement reaction that regenerates the halomethyl starting material.[94] Acyloxymethyl ketones **5.38c** are instead chemically stable compounds, the downside of course is a further reduced reactivity.

Slightly different is the approach of the other two classes of important affinity labels comprising epoxysuccinyl and α,β-unsaturated derivatives (Scheme 5.20). After E-64 (**5.41**) was isolated as a naturally occurring inhibitor of papain in 1978,[95] many analogues have been prepared to study the function of the different groups present.[96] It became evident that the configuration of the epoxide is essential and changes resulted in a consistent decrease in the inhibitory power. Inspired by E-64 it was also envisioned that unsaturated substrate analogues such as **5.43** would have had similar alkylating power acting as nucleophile trapping Michael acceptors. In general these reagents exhibit very specific but somewhat lower inhibitory power due to the slow alkylation reaction, even if showing high affinities.[97] Modification of thiols employing *N*-alkyl maleimides **5.45** is often used in protein sequencing and analysis.[98]



Scheme 5.20 *Epoxysuccinyl and Michael acceptor based inhibitors*

A good part of the inhibitory power of most of the compounds presented is due to the binding affinity of the peptidyl portion of their structure to the active site. It represents the driving force for the formation of the non-covalent enzyme-inhibitor complex as a recognition factor. However, during the alkylation step this part of the inhibitor is already irrelevant and the rate determining step of the inhibition is the

alkylation reaction itself. Therefore, the electrophilicity and leaving ability of the reactive group becomes an important factor.

The use of any of these affinity labels would guarantee the specificity and irreversibility required for the introduction of an artificial cofactor. Maleimide and halomethyl ketone groups seem to be the most reliable alternatives. Eventually, a halomethyl ketone moiety was considered to be the best choice. Its use would allow maintaining generality in the approach, as both serine and cysteine proteases can be alkylated, and probably also other cysteine residues eventually engineered in a given protein. This choice seemed to be a good compromise of reactivity, stability and simplicity of synthesis among the different options. The intrinsic affinity of thiols for these electrophilic reagents would also hopefully allow the design of a cofactor lacking a peptidyl portion.

5.8 Cofactor design

So far we have identified a suitable enzyme whose unique reactive cysteine can be used as chemical recognition site for the irreversible and specific introduction of the artificial cofactor using a halomethyl ketone moiety as linker.

As the most reactive residue of the enzyme is used to covalently immobilize the cofactor, the expectation is that the active site of the enzyme will not directly participate in the new catalysis that will be performed. It will provide water solubility and chiral environment, becoming the scaffold for the transition-metal catalysis to take place. Therefore, the cofactor should have the potential to act as a catalyst without any requirement for the enzyme to participate to the catalytic act in a specific or predetermined way.

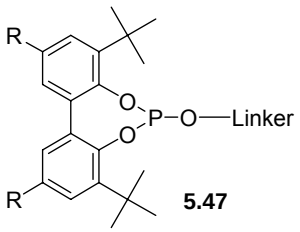
The cofactor, which does not resemble a natural substrate or inhibitor of the enzyme, should possess sufficient water solubility to allow a homogeneous reaction to take place and to overcome the lack of specific binding provided by a peptidyl moiety. The introduction of the cofactor should not alter the structural stability and spatial requirements of the enzyme which could be the cause of possible denaturation or impossibility for the substrate to effectively coordinate to the newly introduced artificial catalytic site.

The design of the cofactor should also provide effective coordination with a metal, small interference of the chemistry involved in its preparation with the reactivity of the labeling moiety and stability of the adduct obtained after enzyme modification. Last but not least, the unfavorable molecular weight ratio between the enzyme and the transition metal requires a highly active transition metal catalyst.

The introduction of an artificial cofactor is meant to broaden the range of catalytic reactions naturally available to enzymes with reactions more characteristic of transition-metal catalysis. Therefore, the core of the cofactor should be constituted by the structure of ligands which, upon coordination with a suitable metal center,

would allow performing reactions like hydrogenation of alkenes,[99] hydroformylation, allylic substitution, hydrosilylation and so on. Phosphorus based ligands are popular for this kind of reactions in combination with Pd, Rh, Ru as metal centers.

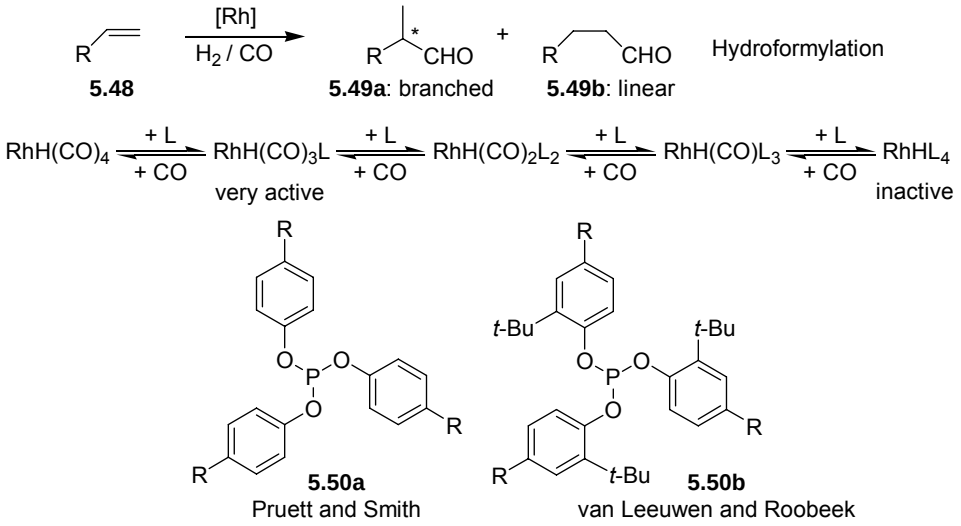
In order to meet as many of these requirements as possible, a bulky achiral monodentate phosphite ligand **5.47** was chosen as core of the cofactor (Figure 5.15). The *t*-butyl substitution and the use of a bisphenol skeleton were chosen for reactivity and stability reasons.



5.47

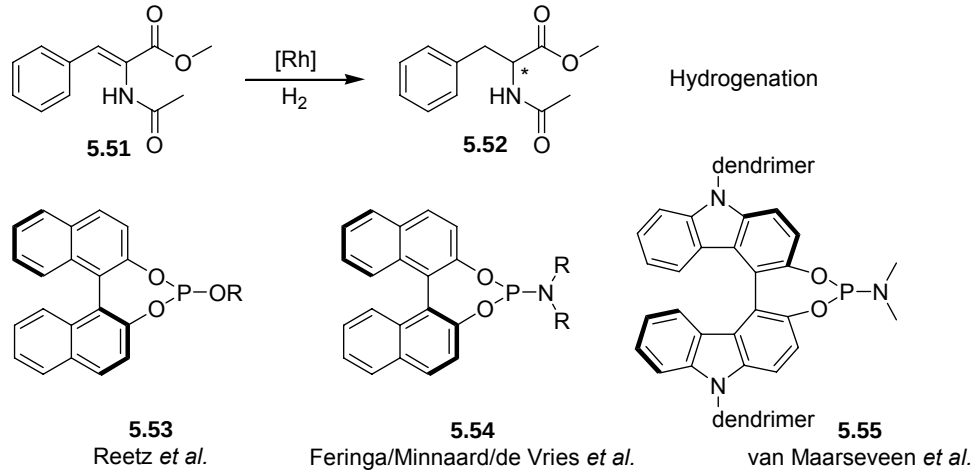
Figure 5.15 *General structure of the core of the cofactor **5.47***

Phosphite ligands have been extensively studied due to their potential in rhodium-catalyzed hydroformylation reactions (Scheme 5.21).[100] The use of monodentate phosphite ligands such as **5.50a**, as effective alternative to phosphines, was first reported in the late sixties by Pruett and Smith in connection with studies conducted by the Union Carbide Corporation (UCC).[101] Further investigations reported by van Leeuwen and Roobeek showed that the increasing steric hindrance of the ligands **5.50b** resulted in higher reaction rates and reactivity toward substrates considered unreactive.[102] It appeared that bulky ligands possess a large cone angle[103] which prevents the coordination of a second ligand. In such a complex the metal center is electron poor which implies a faster dissociation of CO and alkenes coordination and therefore faster catalysis.[104] The same phenomenon has been reported also for Pd-catalyzed aromatic substitution.[105]



Scheme 5.21 *Hydroformylation and early monodentate phosphite ligands*

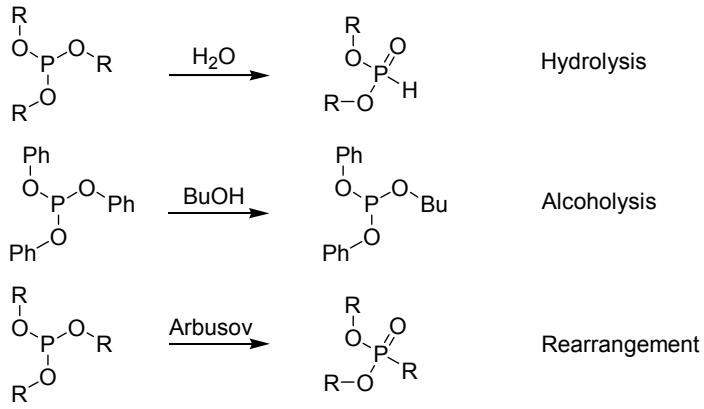
More recently, monodentate phosphite ligands **5.53** have also been introduced in asymmetric Rh-catalyzed hydrogenation reactions by Reetz as well as monodentate phosphoramidite ligands **5.54** by Feringa-Minnaard-de Vries and their coworkers (Scheme 5.22).[6b,c] In this field a less systematic study has been conducted on the possibility and effect of metal unsaturation by coordination of a single monodentate ligand.



Scheme 5.22 *Hydrogenation using monodentate phosphite and phosphoramidite ligands*

Both groups performed hydrogenation reactions in the presence of a 1:1 ratio between metal center and ligand, as in the preliminary investigation of the new systems.[6b,106] The reactions proceeded equally well and in the case of MonoPhos™ (**5.54** where R is a methyl group) a slight increase in reactivity was also observed. Whether the active catalysts contained one or two ligands was, nevertheless, not clear. Rhodium is also known to be able to coordinate up to four equivalents of MonoPhos™ and the resulting catalyst is not active, which means that this ligand is definitely not bulky enough. A study on non-linear effects using MonoPhos™ confirmed that at least under those conditions two ligands were present on the metal center. Recently both groups also showed that it is possible to perform highly enantioselective hydrogenation reactions using a combination of two different ligands.[107] The influence of the additional ligand arises clearly from the results, confirming that the metal center coordinates two ligands if given the possibility. It seems that so far no ligand reported was bulky enough to ensure a 1:1 ratio with the metal center in the complex. Recently, van Maarseveen and coworkers prepared a dendritic version of MonoPhos™ **5.55** (Scheme 5.22). They reported an active catalyst with up to 4 equivalents of ligand and ^{31}P-NMR evidence of the partial formation of a 1:1 complex which means that the ligand was bulky enough to induce metal unsaturation.[108] Unfortunately, no hydrogenation reaction was performed to investigate this possibility. The introduction of the phosphite cofactor **5.47** in the active site of papain is an excellent opportunity to use a ligand that will provide a 1:1 catalytic species with the metal center, as the rhodium is expected to be able to coordinate only one enzyme-cofactor adduct due to steric hindrance. Moreover, the catalyst should be active enough to overcome the unfavorable molecular weight difference between the enzyme and the catalytically active center.[109]

Although less prone to oxidation than phosphines, phosphites might undergo few side reactions which cause their degradation and the most frequently occurring ones are depicted in Scheme 5.23.[110]



Scheme 5.23 *Possible phosphite decomposition pathways*

Arbusov rearrangements are usually metal-catalyzed and typical for aliphatic phosphites. A systematic study conducted by Billig and coworkers (UCC) to understand the causes of ligand decomposition showed that bulky phosphite ligands such as **5.50c**, besides providing very reactive catalysts, are also less prone to hydrolysis.[111] The stability seems to further increase when using bisphenols like **5.56a-b** (Figure 5.16).

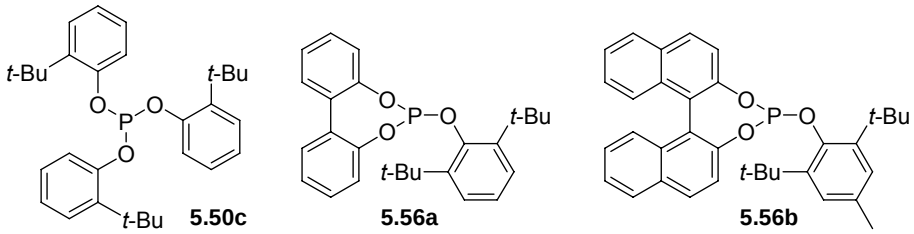


Figure 5.16 *Bulky phosphites are more resistant to hydrolysis*

5.9 Cofactor synthesis

The size of the phosphite ligand **5.47** was reduced as much as possible to minimize water solubility problems. The first two cofactors **5.47a-b** synthesized are depicted in Figure 5.17.

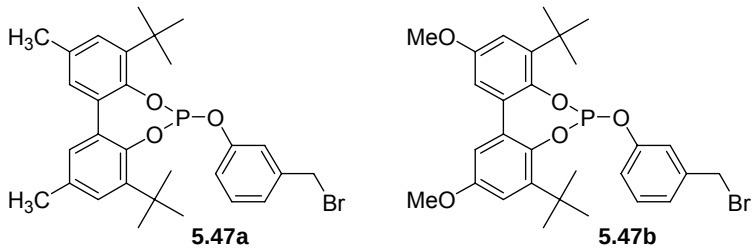
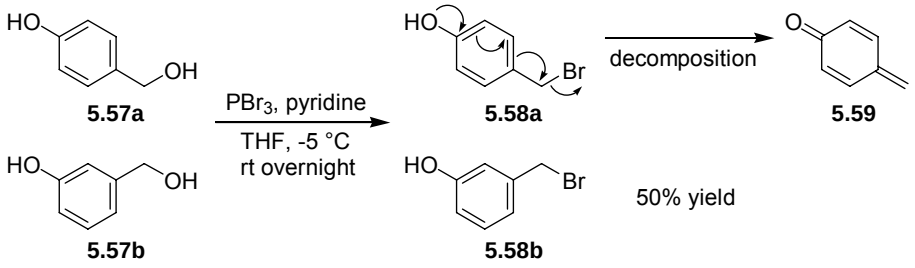


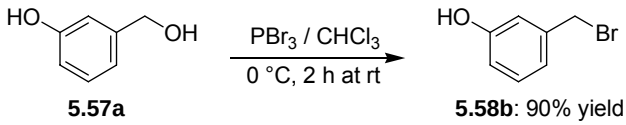
Figure 5.17 *Phosphite based cofactors **5.47a** and **5.47a** prepared*

A bromobenzyl phenol was at first considered as labeling moiety in order to keep the linker as short as possible. The alkylation was expected to be the result of the nucleophilic attack of Cys-25 to the bromide being the leaving group. Alkylation of papain using a benzylic bromide was also reported by Kaiser and Levine (Scheme 5.2).[21] Among the different halides, bromide was chosen because of its intermediate reactivity (I>Br>Cl) but also better stability toward degradation than iodide. In order to have a possible variation both 3-bromobenzyl (**5.58a**) and 4-bromobenzyl (**5.58b**) phenols were prepared following a literature procedure starting from the corresponding hydroxy-benzyl alcohols **5.57a-b** as shown in Scheme 5.24.[112]



Scheme 5.24 *Synthesis of bromobenzyl phenols **5.58a-b***

A solution of hydroxyl-phenol **5.57a-b** in dry THF was added dropwise at -5 °C to another THF solution containing PBr_3 and pyridine. The reaction mixture was left at room temperature overnight and then filtered over celite. After removal of the solvent, toluene was added; the solution was maintained at -20 °C for a couple of hours and filtered again over celite. In the literature procedure, products **5.58a** and **5.58b** are reacted without further purification as a toluene solution that can be stored in the fridge. However, both solutions started to darken and purification was attempted. Column chromatography allowed the isolation of **5.58b** as an oil solidifying on standing. The solution of **5.58a** could not be purified because of the presence of considerable amount of dark and insoluble material. The decomposition of **5.58a** seems to be caused by easy HBr elimination and formation of *p*-quinone methide (**5.59**).[112] More recently *p*-quinone methide (**5.59**) has been reported to be a short-lived reactive species, which might explain the formation of insoluble degradation products.[113] Due to the clear instability, the synthesis of **5.58a** was abandoned and a slightly different literature procedure was used to obtain **5.58b** (Scheme 5.25).[114]

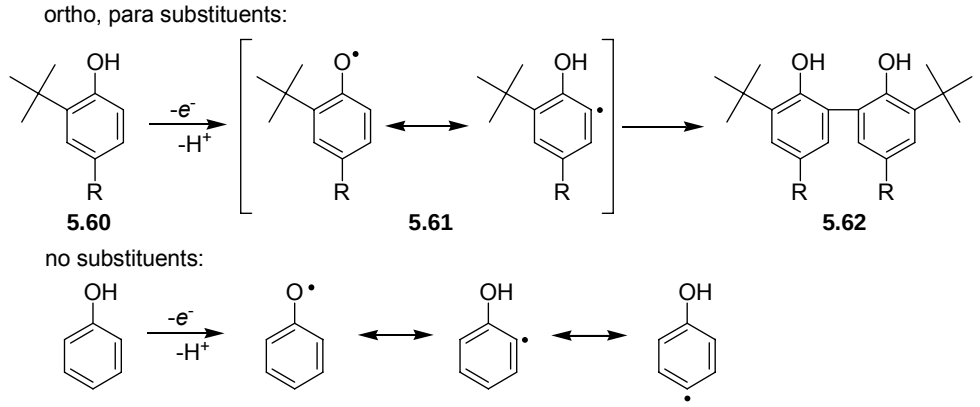


Scheme 5.25 *Improved synthesis of bromo-phenol **5.58b***

The main difference in the procedure, besides the absence of base, is the reverse addition of PBr_3 to a suspension of alcohol in $CHCl_3$. After addition of water, extraction and removal of the organic solvent, **5.58b** was obtained pure in 98% yield and could be used and stored in the fridge without further purification. However, due to the yellowish color it was eventually purified by column chromatography and it was recovered in 90% yield as a colorless oil solidifying on standing.

The bisphenols **5.62** were prepared according to literature procedures based on the oxidative dimerization of the corresponding phenols **5.60** (Scheme 5.26). The tendency of phenols to oxidation has been extensively studied as they are considered important antioxidants. There is quite an amount of early literature

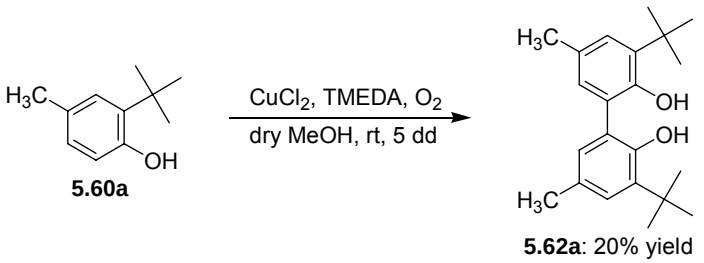
dealing with the response of differently substituted phenols to various oxidizing agents.[115]



Scheme 5.26 *The presence of substituents limits the oxidation products*

In all the procedures adopted, the first step seems to be the abstraction of the phenolic hydrogen of **5.60** and the oxidation is a one electron process. The radical **5.61** formed is stabilized by resonance in *ortho* and *para* positions and the reactivity of these three positions is responsible for the potentially wide number of products. Out of all the oxidation products it is possible to mainly obtain symmetric bisphenols **5.62** depending on the substitution. Bulky groups in *ortho* and *para* positions limit the number of oxidation products and polymer formation.[116]

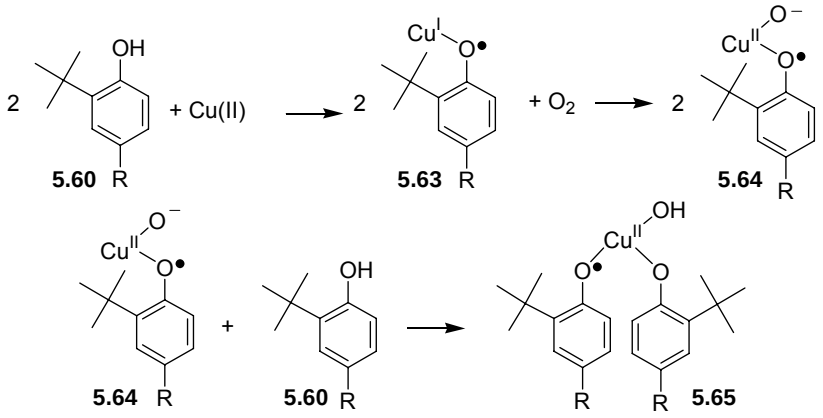
The first procedure applied involved the use of $CuCl_2$ (0.5%) in the presence of TMEDA (1:2) under an atmosphere of oxygen (Scheme 5.27).[117] The reaction was rather slow and was eventually stopped after 5 days providing **5.62a** from **5.60a** in a disappointing 20% yield after column chromatography.



Scheme 5.27 *Synthesis of bisphenol **5.62a** using Cu(II)-diamine complex*

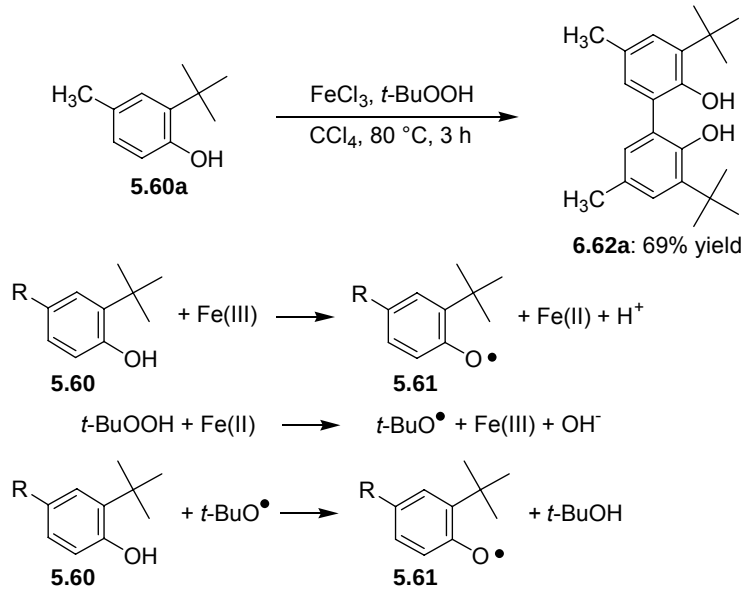
Analysis of the ^{1}H-NMR crude of the reaction mixture showed mainly the presence of product **5.62a** and starting material **5.60a**. Two other oxidation side compounds were clearly visible on TLC but present in small amount. The use of copper-amine complexes is reported to be important not only in the initiation of the reaction but

also for the coupling pattern and the reaction was proposed to proceeds by coordination of the reagent to the complex (**5.63** and **5.65**), as shown in Scheme 5.28.[118,119]



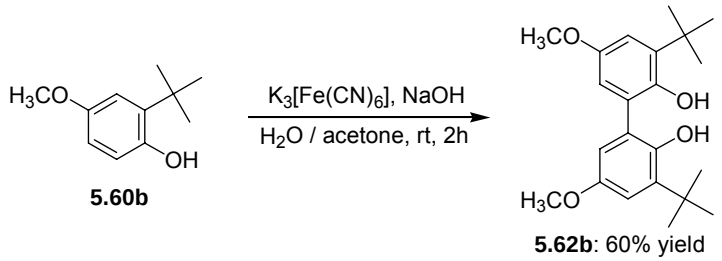
Scheme 5.28 *Some intermediates using Cu(II)-diamine complex*

In retrospect, a faster reaction following this procedure might have been obtained by increasing the amount of catalyst, as elsewhere it was reported that using similar Cu-diamine complexes the reaction rate had a linear dependence with the concentration of the catalyst.[118] Bubbling of molecular oxygen directly in the reaction solution might have also increased the reactivity, improving its solubility in the reaction media. A higher yield (69%) of the desired bisphenol **5.62a** was obtained by using $FeCl_3$ (25%) in the presence of *t*-BuOOH under a nitrogen atmosphere, as described in a literature procedure.[120]



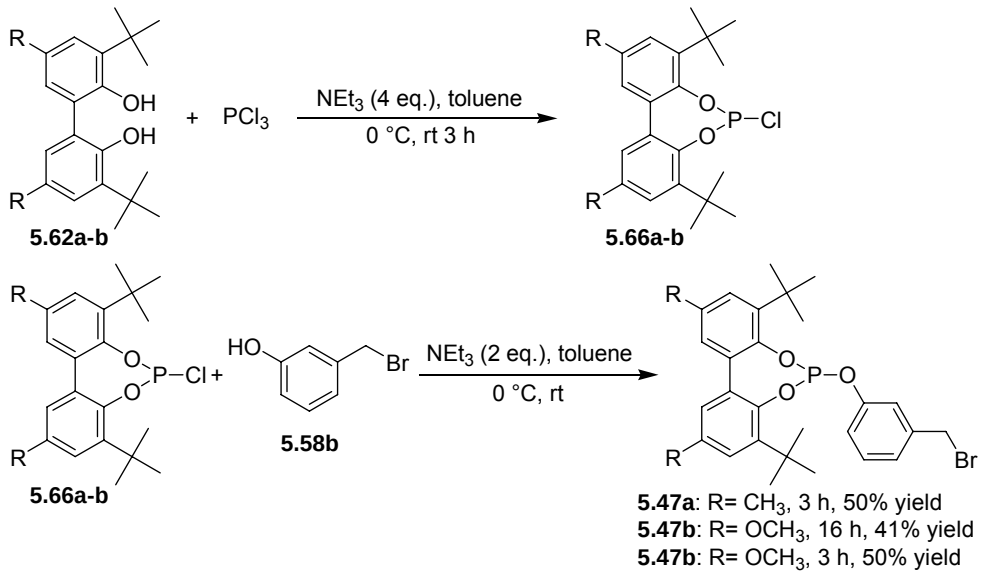
Scheme 5.29 *Synthesis of bisphenol **5.62a** using Fe(III)*

In the third procedure used for the preparation of **5.62b**, alkaline ferricyanide was employed as oxidizing agent and the product was isolated in 60% yield after recrystallization (Scheme 5.30). In this case, an equimolar amount of Fe(III) is necessary as the obtained Fe(II) cannot be oxidized again to Fe(III) in *situ*.[121]



Scheme 5.30 *Synthesis of bisphenol **5.62b** with equimolar amount of Fe(III)*

Following a modified literature procedure reported by van Leeuwen and coworkers,[122] bisphenols **5.62a-b** were used to prepare the corresponding phosphorus chlorides **5.66a-b** by reacting them with PCl_3 in the presence of base using toluene as solvent. The reaction of **5.66a-b** with benzyl bromide **5.58b** delivered the desired phosphites **5.47a-b** (Scheme 5.31).



Scheme 5.31 *Synthesis of phosphites **5.47a** and **5.47b***

Phosphorus trichloride was added dropwise to a solution of NEt_3 in dry toluene at 0 °C. A toluene solution of bisphenols **5.62a** or **5.62b** was also added dropwise and the mixture was stirred at room temperature. The progress of the reaction was monitored by ^{31}P-NMR following the disappearance of the starting material signal (202 ppm) and the appearance of the phosphorus chloride **5.66a-b** signals (~172 ppm). During the preparation of **5.47a**, when PCl_3 had disappeared, another 2 equivalents of NEt_3 were added to the reaction mixture at 0 °C, followed by a toluene solution of benzyl phenol **5.58b**. The reaction was again checked by ^{31}P-NMR following the appearance of product **5.47a** (~138 ppm). Work up consisted of the filtration of the triethylamine salts after the addition of Et_2O and removal of the solvent. Phosphite **5.47a** was obtained in 50% yield after purification by column chromatography in the presence of 0.5% of NEt_3 and under nitrogen atmosphere using distilled solvents. Column chromatography performed in dry and slightly basic conditions have been reported to minimize the degradation of phosphites during the purification step.[110] A slightly different procedure was used for the preparation of phosphite **5.47b**. The triethylamine salts formed during the first step were removed by quick filtration under nitrogen atmosphere and volatiles removed before adding benzyl phenol **5.58b**. The resulting reaction mixture was stirred overnight and the product **5.47b** was isolated in 41% yield. It was not clear if the lower yield should be attributed to the intermediate salts filtration or to the longer reaction time which could have compromised the stability of the phosphite or of the benzyl bromide. However, when the second step of the reaction was stopped after 3 h, **5.47b** was isolated in 50% yield after column chromatography using again

distilled solvents but without the presence of NEt_3. Both compounds were stored at -12 °C.

5.10 Cofactor solubility tests

Although keeping the size of the cofactor as small as possible, phosphites **5.47a-b** were assumed to be fairly hydrophobic molecules and solubility in water was already considered to be an issue. It was desirable to be able to dissolve enough cofactor in aqueous solution to obtain a good inhibition profile of papain. A few organic solvents miscible with water were screened in order to find the best compromise between the use of a minimum amount of organic solvent, which would avoid possible destabilization of papain, and best solubility of the cofactor. The tests were first performed on **5.47a**.

This test was based on visual observation of turbidity of the solution containing the cofactor in different mixtures of organic solvents in water. At first a 3.7 mM stock solution of **5.47a** in CH_3CN was prepared and 50 µL of this solution were added to the water / organic solvent solutions obtaining a final concentration of ligand of 180 µM. The results of the test are shown in Table 5.2.

Table 5.2 *Solubility of **5.47a** in water / organic solvents mixtures*

solvent	volume (%) in H_2O	solution appearance
CH_3CN	10	quite turbid
DMSO	10	turbid / precipitate
DMF	10	turbid / precipitate
DMF	20	turbid / precipitate
*i*PrOH	10	turbid
*i*PrOH	20	almost clear
acetone	10	quite clear
1,4-dioxane	10	clear
MeOH	50	quite clear

After this first qualitative examination, 1,4-dioxane appeared to be the best solvent. Therefore, it was decided to test different concentrations of **5.47a** in different percentages of solvent adding it to a 100 mM phosphate buffer solution at pH 7 that will be used in all papain manipulations. Unfortunately, it became immediately clear that, using buffered aqueous solutions, the cofactor **5.47a** was not soluble enough anymore and started precipitating. Therefore, the test was performed starting from a more diluted 1 mM stock solution of **5.47a** and changing the final percentage of 1,4-dioxane present in the buffered solution from 5% up to 50%.

Moreover, analysis of the UV-Vis spectra of **5.47a** in the different solutions was also diagnostic as turbidity resulted in disturbed baselines. Final phosphite concentrations of 50 μM, 25 μM and 12.5 μM, respectively, were considered and the resulting UV profiles obtained for the highest concentration used are shown in Figure 5.18.

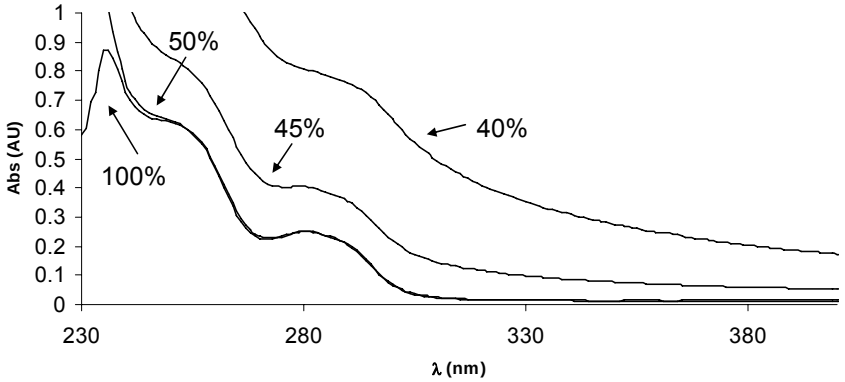


Figure 5.18 *Uv spectra of phosphite **5.47a** (50 μM) in 100 mM phosphate buffer with different amount of 1,4-dioxane*

At a final cofactor concentration of 50 μM, a clear solution was obtained using 50% 1,4-dioxane in buffered solution. The UV-Vis spectra showed that with 45% organic solvent the solution started to be slightly turbid but it could be considered still acceptable, whereas a lower amount of 1,4-dioxane (40%) was clearly too little. Similarly, it was found that clear solutions could be obtained using 40% of 1,4-dioxane if the final concentration of **5.47a** was 25 μM and 30% for 12.5 μM.

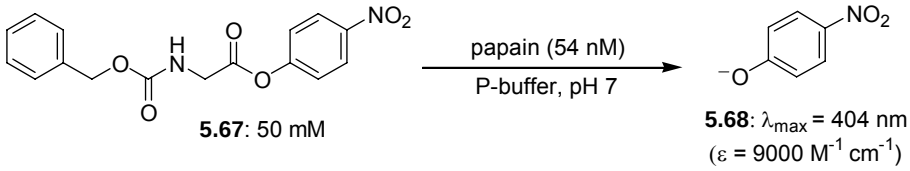
A similar test was performed with phosphite **5.47b**, which was prepared in the hope that it would have better solubility in water; however, it behaved exactly in the same way, so **5.47b** was no longer considered in this investigation.

5.11 Papain pretreatment and activity test

Papain is commercially available as a buffered aqueous suspension in 0.05 M sodium acetate at pH 4.5 and containing 0.01% of thymol.[123] Papain needs to be activated using reducing agents such as cysteine, dimercaptopropanol or DTT (dithiothreitol). The mechanism and the reason for this required procedure are not well understood.[124] The use of thiol-based reducing agents is a standard protocol when using papain and the source of inactivation seems to be directly connected with the way in which the enzyme is produced.[125] More insight into this subject can be found later in this chapter (page 215).

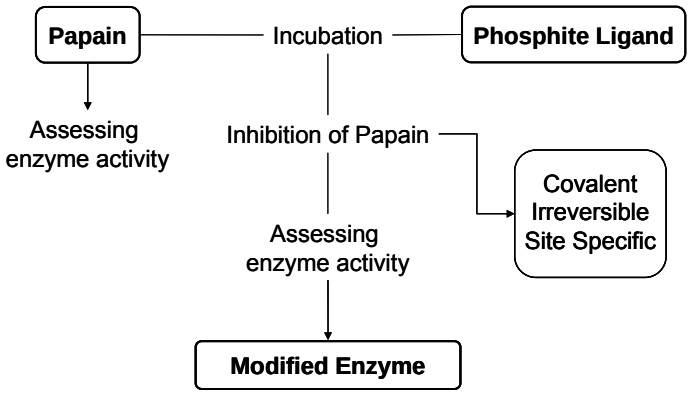
Activated papain solutions usually contain stabilizing agents such as EDTA and the previously mentioned reducing agents, used to avoid inactivation of the enzyme by heavy metal complexation or oxidation, respectively. For the same reasons, the buffered solution should be prepared using bidistilled water and all manipulations should be performed using carefully degassed solutions. Adapting a protocol reported by Albeck and Kliper, papain was activated by incubation at room temperature in 100 mM potassium phosphate buffer at pH 7.0, in the presence of DTT and EDTA, for 20 minutes.[126]

Another advantage in using an enzyme as protein scaffold for this study is the possibility of monitoring the progress of the cofactor introduction using standard activity tests which measure the hydrolytic activity of the enzyme (Scheme 5.32).



Scheme 5.32 *Papain activity test using **5.67** as standard substrate*

The introduction of the cofactor **5.47a** by covalent modification of Cys-25 results in the inhibition of papain that will no longer be able to hydrolyze ester or amide bonds. Conveniently, ester **5.67** (used as test substrate) upon hydrolysis releases a *p*-nitrophenolate (**5.68**) that can be easily monitored by UV-Vis absorption spectroscopy.[126,127] This test was used to assess papain activity after the activation and in different moments during the introduction of **5.47a** to check the progress of the inactivation. Upon incubation with **5.47a**, the inability of papain to hydrolyze the standard substrate **5.67** would provide the first evidence that the alkylation proceeded successfully (Scheme 5.33).



Scheme 5.33 *Assessment of papain activity as the first proof of successful alkylation*

There were some concerns that the addition of a too high percentage of organic solvent might compromise the stability or reactivity of papain. Therefore, the hydrolysis of Z-Gly-*p*-NPE (**5.67**) was used to determine the influence of 1,4-dioxane on the enzyme catalysis. To avoid the inactivation of papain due to oxidation of the reactive Cys-25 also 1,4-dioxane was carefully degassed and a protocol compatible with the UV-Vis analysis was prepared.

Table 5.3 *Composition of solution A used for papain activation*

	volume	final concentration
P-buffer (100 mM)	976 µL	-
DTT (0.1 M)	10 µL	1 mM
EDTA (0.5 M)	4 µL	2 mM
papain (1.1 mM)	10 µL	11 µM

An aliquot (50 µL) of the solution used for the activation (solution A, Table 5.3) was diluted to 1 mL with phosphate buffer and the required amount of 1,4-dioxane (solution B). A sample (90 µL) of this dilution solution was added to a quartz cuvette containing phosphate buffer (890 µL). The substrate **5.67** (20 µL, acetone) was added as last to this activity test solution C and its hydrolysis was followed by UV. The results shown in Figure 5.19 were quite surprising.

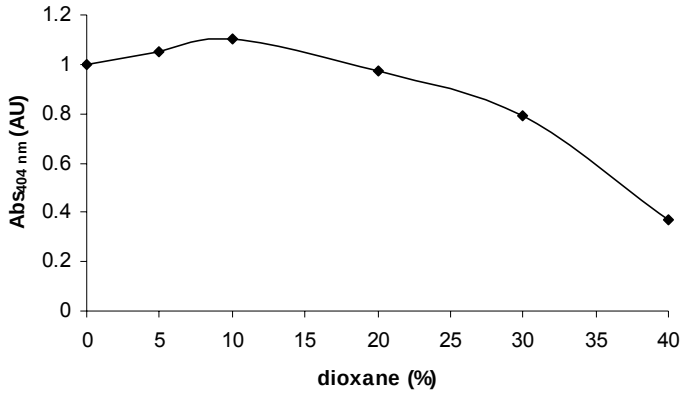


Figure 5.19 *Effect of organic solvents on papain activity: normalized p-nitrophenolate (**5.68**) absorbance (λ= 404 nm) after 10 min. reaction vs. 1,4-dioxane (%) present in solution.*

The addition of 5% or 10% of 1,4-dioxane resulted in a slightly higher value for the absorbance of the *p*-nitrophenolate (**5.68**) released during the hydrolysis, which was considered an artifact due to interactions with the organic solvent. However, increasing the amount of 1,4-dioxane considerably decreased the hydrolytic

activity of papain. In another set of experiments papain, after the addition of 5% or 20% of 1,4-dioxane, was either immediately used or first incubated for half an hour in the presence of the solvent. As a result, the incubated papain was respectively 30% and 43% less active than when directly used. The increasing presence of 1,4-dioxane could have destabilized and therefore inactivated the enzyme on a structural level. However, when a decrease in activity was noticed also using 5% organic solvent followed by an incubation period, the possibility that 1,4-dioxane was contaminated with potential inactivating agents started to arise. It was found that commercial 1,4-dioxane might contain acetaldehyde, ethylene acetal and peroxides.[128] All these compounds might indeed form adducts or oxidize the Cys-25 thiol and consequently deactivate the enzyme. Purification of 1,4-dioxane by percolation through a column of activated alumina (80 g per 100-200 mL) efficiently solved the problem. This was proven by following the hydrolysis of **5.67** with and without 50% of purified 1,4-dioxane and the release of *p*-nitrophenolate (**5.68**) showed a very similar profile in both cases (Figure 5.20).

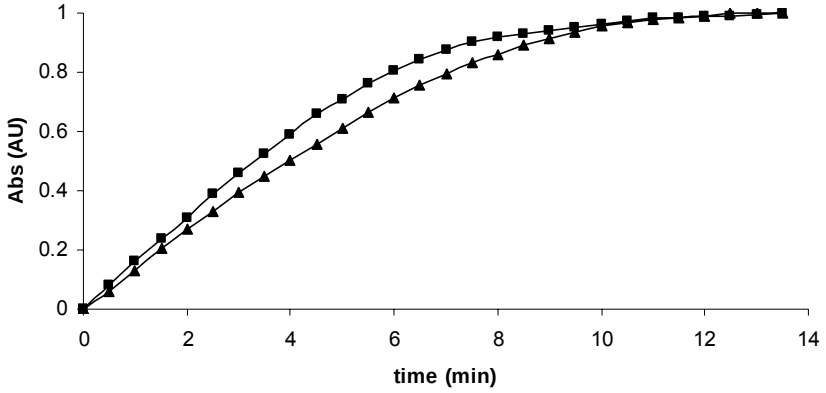


Figure 5.20 *Hydrolysis of **5.67** catalyzed by papain followed by monitoring the release of p-nitrophenolate (**5.68**) by UV-Vis absorption spectroscopy (λ= 404 nm) with (▲) or without (■) the presence of 50 vol% purified 1,4-dioxane.*

Alternatively, 1,4-dioxane was also distilled from sodium under N_2 atmosphere. In both cases, the purified solvent was directly used or stored at 4 °C. This allowed the use of a phosphate buffer containing 50 vol% 1,4-dioxane and therefore a higher concentration of cofactor **5.47a** during the alkylation procedure.

5.12 Alkylation of papain using linker 5.58b and cofactor 5.47a

The linker part of cofactor **5.58b** was initially used as model for the preparation of the inhibition protocol and to test the inhibitory power of the labeling compound itself. In the protocol used to test the influence of 1,4-dioxane on papain the dilution

step was transformed into an incubation step as shown in Table 5.4. As before, a sample (90 µL) of this incubation solution B was used in the activity test for the hydrolysis of Z-Gly-*p*-NPE (**5.67**).

Table 5.4 *Composition of incubation solution B*

	volume	final concentration
P-buffer (100 mM)	450 µL	-
papain (solution A)	50 µL	0.55 µM
5.58b (dioxane)	500 µL	0.5, 2.3, 4.6 mM

At first, the incubation of the activated papain (0.55 µM) with the linker **5.58b** (0.5 mM) was performed at room temperature. The extent of the inhibition was checked by comparing the ability of papain in catalyzing the hydrolysis of **5.67** before and during the incubation with **5.58b** (Figure 5.21).

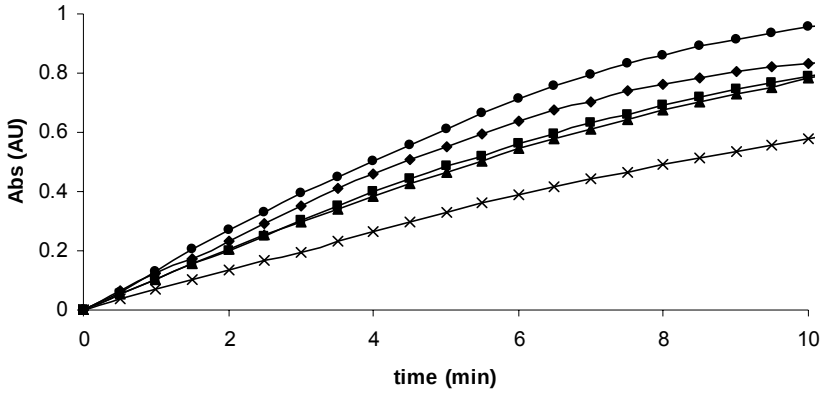


Figure 5.21 *Hydrolysis of **5.67** catalyzed by papain followed in time by monitoring the release of p-nitrophenolate (**5.68**) by UV-Vis absorption spectroscopy (λ= 404 nm): before (●) and after incubation of papain at rt with **5.58b** (0.5 mM) after 0:30 h (♦), 1:20 h (■), 2:15 h (▲), overnight (x).*

The alkylation seemed to proceed very slowly during the first couple of hours and it was decided to continue the incubation overnight. However, as shown in Figure 5.21, even after one night in the presence of **5.58b**, papain appeared to be still quite active in catalyzing the hydrolysis of **5.67**. Therefore, in another set of experiments, maintaining 50% of 1,4-dioxane as cosolvent, papain was incubated at room temperature for 3 h in the presence of an increased amount of labeling compound **5.58b** (2.3 and 4.6 mM), anticipating that faster inhibition might occur. Papain was also incubated for one hour in the presence of the same concentrations of **5.58b** but at higher temperature (37 °C). A control was also prepared in which papain was left at 37 °C in the absence of the labeling

compound **5.58b**, in order to check if an increased temperature was compromising the catalytic activity of the enzyme. The influence of the different conditions was once more indirectly monitored following the catalyzed hydrolysis of **5.67**. The residual catalytic activity of papain after incubation under the different conditions is shown in Figure 5.22 in relation with the untreated and fully active enzyme.

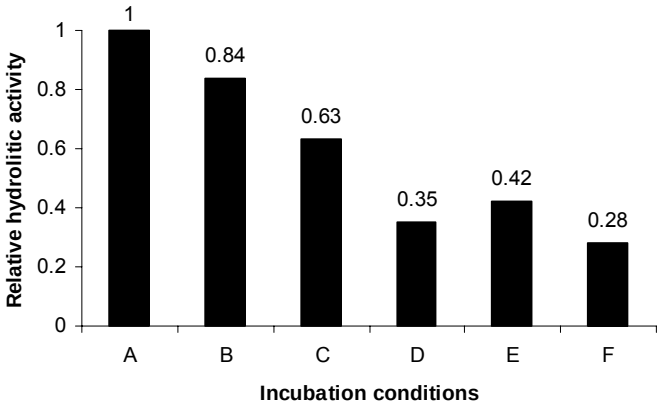


Figure 5.22 *Maximum release of p-nitrophenolate (**5.68**) measured by UV-Vis absorption spectroscopy (λ= 404 nm) due to the hydrolysis of **5.67** catalyzed by papain: **A**) without any treatment used as reference; **B**) after 1 h at 37 °C; **C**) after 3 h incubation with 2.3 mM **5.58b** at rt; **D**) after 1 h incubation with 2.3 mM **5.58b** at 37 °C; **E**) after 3 h incubation with 4.6 mM **5.58b** at rt; **F**) after 1 h incubation with 4.6 mM **5.58b** at 37 °C.*

The incubation of papain in the presence of a higher amount of labeling reagent **5.58b** (4.6 mM) at 37 °C induced a reduction of the release of *p*-nitrophenolate (**5.68**) of around 70% (Figure 5.22, **F**). However, under the same conditions even without **5.58b** a considerable decrease of activity was also observed (**B**). Alkylation of the enzyme at room temperature seemed to be a more reliable procedure, as it would avoid deactivation not related to the introduction of the cofactor.

At this point the complete phosphite cofactor **5.47a** was also tested in the alkylation of papain. Due to the lower solubility of **5.47a** compared to the labeling reagent **5.58b**, a lower concentration of 0.5 mM in 50% 1,4-dioxane was used. The solution was incubated for two nights and checked for activity after each night. In one experiment untreated papain was also left at room temperature for two nights. The solutions were tested for residual enzymatic activity as previously described and the resulting profiles of the release of *p*-nitrophenolate (**5.68**) are depicted in Figure 5.23.

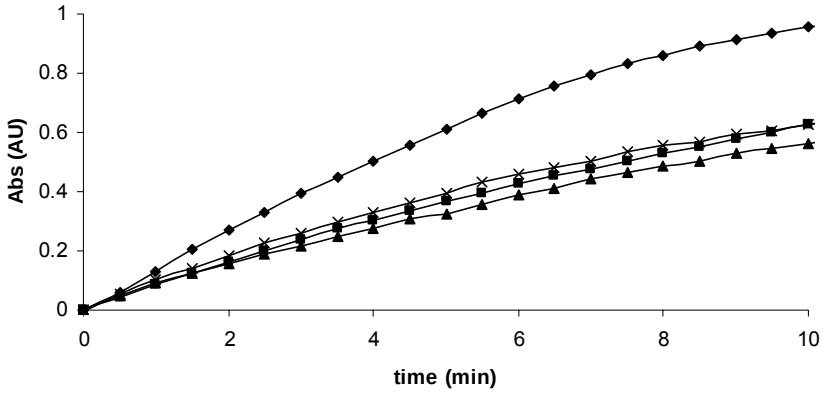


Figure 5.23 *Profiles of p-nitrophenolate (**5.68**) release upon hydrolysis of **5.67** in the presence of papain: untreated (♦), after 1 night incubation with 0.5 mM **5.47a** (x), after 2 nights incubation with 0.5 mM **5.47a** (▲), after 2 nights without **5.47a** (■).*

After one night incubation with **5.47a**, the test showed a residual activity comparable to the one observed for the linker **5.58b** under the same conditions (Figure 5.21). This gave the impression that at least the complete cofactor was accepted as much as the smaller linker. However, papain showed very little decrease in reactivity after one extra night and a comparable decrease in activity was observed also for papain incubated at room temperature for two nights even without the presence of the inhibitor **5.47a**. The process was definitely too slow and did not guarantee completion, whereas, the poor solubility of **5.47a** in phosphate buffer did not allow to reach higher concentrations, which anyway did not show to improve the results even when using **5.58b**.

Prolonged incubation times and higher temperatures seemed to hamper the intrinsic activity of papain and were not particularly beneficial for the alkylation process. Furthermore, shorter reaction times would also preserve the stability of the phosphite cofactor **5.47a** itself. In order to achieve a more efficient alkylation protocol both the reactivity of the labeling moiety and the solubility of the cofactor had to be improved, which made it necessary to redesign the phosphite cofactor.

5.13 Improvements in the cofactor design

In order to improve the solubility of the cofactor in buffered solutions, the bisphenol skeleton was equipped with two triethyleneglycol tails (Figure 5.24). Polyether-substituted phosphorus ligands have been used on a few occasions in Rh-catalyzed hydrogenation[129] and hydroformylation[130] reactions in order to improve

their water solubility as an alternative to the more commonly used amino, carboxylic acid, hydroxy and sulfonate groups.[131]

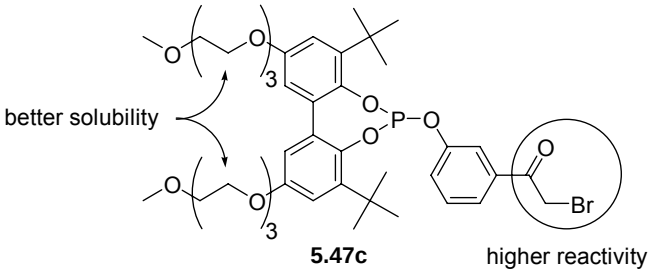
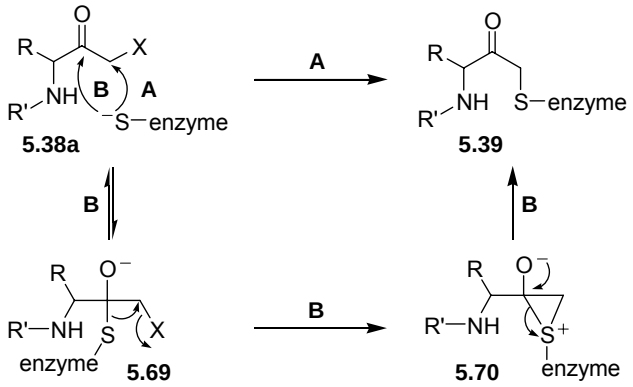


Figure 5.24 *Improved design for the new cofactor **5.47c***

The labeling benzyl bromide moiety was also abandoned and it was substituted with a potentially more reactive bromomethyl ketone. As shown in Scheme 5.34, there are two possible pathways for the mechanism of inhibition of cysteine proteases whilst using halomethyl ketones.[65]

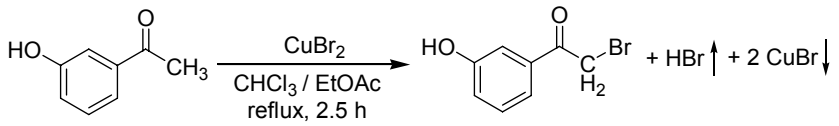


Scheme 5.34 *Mechanism of inhibition using halomethyl ketones **5.38a***

According to pathway A, Cys-25 would react directly with the carbon of the halomethyl group of **5.38a** in a nucleophilic substitution fashion directly providing the thioether **5.39**. The same reaction is expected whilst using the benzyl bromide **5.58b** and the corresponding phosphite cofactor **5.47a**. Instead of following pathway B, Cys-25 would first attack the carbonyl group of **5.38a** with the subsequent formation of a tetrahedral intermediate **5.69** which is trapped as a sulfonium ion intermediate **5.70**, finally affording the identical product **5.39**. Following the classification previously provided about inhibitors (Figure 5.14) halomethyl ketones **5.38a** should then be better defined as mechanism based inhibitors than affinity labels, since the formation of a tetrahedral intermediate is part of the proteases catalytic pathway. According to the literature, pathway B seems to be the most accepted, however, experimental proof for the existence of

adducts like **5.69** has been only provided for trypsin which is a serine and not a cysteine protease.[132] In this respect, it should have also been considered that in the case of serine proteases the irreversible inhibition is the result of the alkylation of the catalytic histidine and not of the serine, which makes the comparison not consistent. This does not exclude nucleophilic substitution on a benzyl bromide; however, the use of a halomethyl ketone might result in a faster reaction, especially if the carbonyl moiety is also involved in hydrogen bonding with some of the protein residues as shown in Figure 5.11.

The new affinity label 3-hydroxy-phenacyl bromide (**5.72**) was conveniently prepared following a literature procedure as depicted in Scheme 5.35.[133]

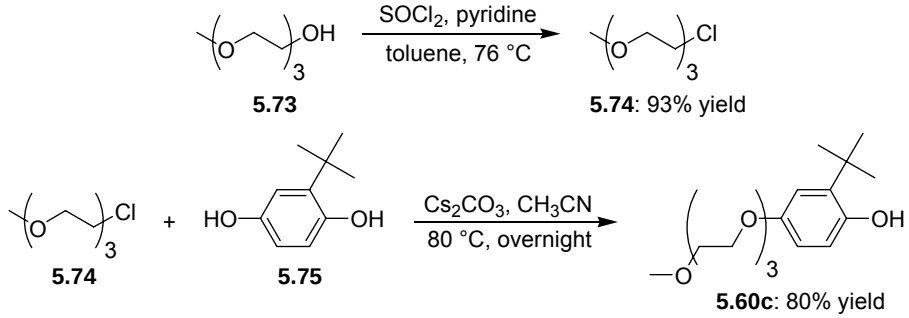


5.71 **5.72**: 85% yield

Scheme 5.35 *Synthesis of 3-hydroxy-phenacyl bromide (**5.72**)*

The heterogeneous reaction consisted in the bromination of 2-hydroxy acetophenone (**5.71**) with $CuBr_2$ and was very convenient as the by-products of the reaction were either volatile (HBr) or could be filtered at the end of the reaction (CuBr). The procedure was selective for one α-bromination, as the presence of over brominated products was not observed by ^{1}H-NMR. The α-bromo ketone **5.72** was obtained in a good 85% isolated yield after column chromatography. The lachrymatory compound **5.72** was preferably stored at 4 °C.[134]

Unlike the bisphenols **5.62a-b** previously prepared, in this case the required starting material was not commercially available. Phenol **5.60c** was obtained by monoalkylation of 2-*t*-butyl-hydroquinone (**5.75**) with the chlorinated derivative of triethyleneglycol **5.74** as depicted in Scheme 5.36.

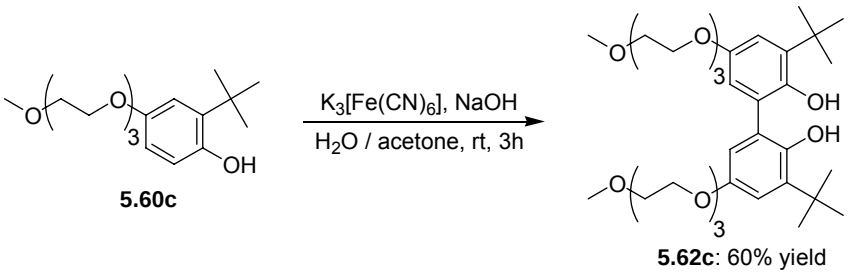


5.73 **5.74**: 93% yield

5.74 **5.75** **5.60c**: 80% yield

Scheme 5.36 *Synthesis of **5.60c** by monoalkylation of 2-t-butyl-hydroquinone (**5.75**) with 1-[2-(2-chloro-ethoxy)-ethoxy]-2-methoxy-ethane (**5.74**)*

The chlorination of methoxy triethyleneglycol **5.73** was achieved by reaction with thionyl chloride in the presence of pyridine. The desired product **5.74** was obtained in 93% yield after work up and was used without any further purification. A modification of a literature procedure reported by Stoddard and coworkers for the preparation of polyether macrocycles was used for the monoalkylation step.[135] In the original procedure to achieve monoalkylation a huge excess of a hydroxy phenol was reacted in acetonitrile in the presence of K_2CO_3 as base. As depicted in Scheme 5.36, in this case the chlorinated triethyleneglycol derivative **5.74** was reacted with a small excess (1.2 equivalents) of 2-*t*-butyl-hydroquinone (**5.75**) and Cs_2CO_3 was used as inorganic base.[136] The expectation was that, due to the bulkiness of the *t*-butyl substituent on the hydroquinone **5.75**, reaction at the less bulky hydroxy group would be preferred. It was pleasing to see that the assumption proved to be correct and the desired product **5.60c** was obtained in 80% yield. At this point, bisphenol **5.62c** was prepared using one of the previously described oxidative dimerization procedures involving the use of a stoichiometric amount of Fe(III) and it was obtained in 60% yield as a thick yellowish oil (Scheme 5.37).



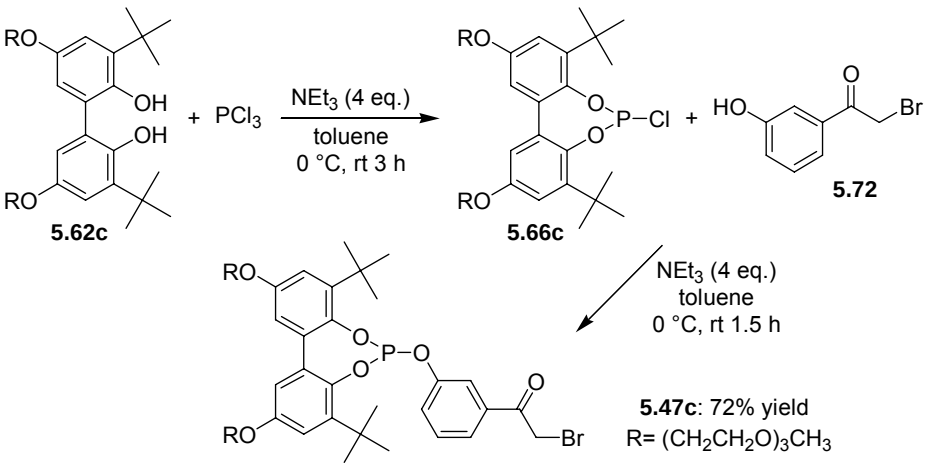
5.60c

5.62c: 60% yield

Scheme 5.37 *Synthesis of bisphenol **5.62c** by oxidative dimerization of **5.60c***

Finally, bisphenol **5.62c** and PCl_3 were reacted in dry toluene in the presence of NEt_3 for 3 hours. Quick filtration of the salts after addition of dry ether and removal of the solvents afforded the crude chloro phosphite **5.66c** that without further purification was used in the following step (Scheme 5.38). Similarly to the first step of the synthesis, chloro phosphite **5.66c** was reacted with **5.72** in the presence of NEt_3 using toluene as solvent. The progress of the reaction was followed by ^{31}P-NMR and at the disappearance of the phosphorus chloride signal (172 ppm) the reaction was stopped. Phosphite **5.47c** was directly purified and obtained in 72% yield after column chromatography and was stored at -12 °C.

The stability of phosphite cofactor **5.47c** in the presence of water was checked by ^{31}P-NMR (137.6 ppm). No degradation of the ligand was detectable even after 1 week in a 1:1 mixture D_2O/acetone.



Scheme 5.38 *Synthesis of phosphite **5.47c***

5.14 Alkylation of papain using linker 5.72 and cofactor 5.47c

In two separate experiments, activated papain was incubated for one hour in the presence of the labeling compound **5.72** or the phosphite cofactor **5.47c**, respectively. The only difference with the protocols previously used was the presence of only 5% 1,4-dioxane. The residual activity of the enzyme was assessed following the catalyzed hydrolysis of **5.67** by UV, as previously described and the hydrolysis profiles are depicted in Figure 5.25.

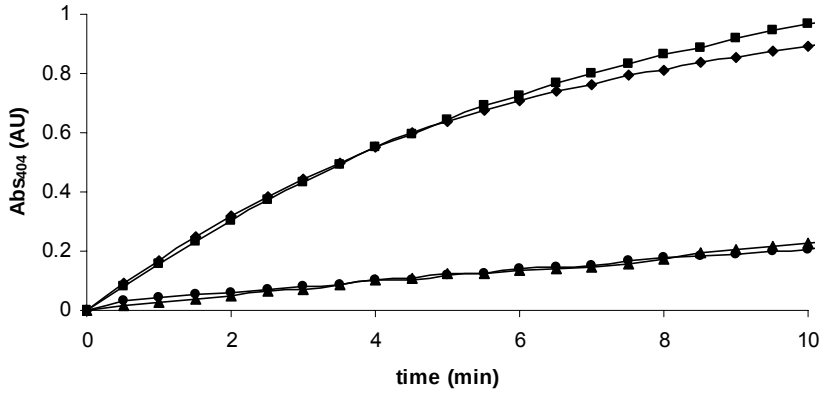


Figure 5.25 *Hydrolysis of **5.67** catalyzed by papain followed in time by monitoring the release of p-nitrophenolate (**5.68**) by UV-Vis absorption spectroscopy (λ= 404 nm) after 1h incubation: without 1,4-dioxane (♦); with 5% 1,4-dioxane (■); with 50 μM **5.72** (▲); with 50 μM **5.47c** (●).*

It was pleasing to see that papain showed almost no activity after only one hour incubation in both experiments with the affinity label **5.72** and the phosphite cofactor **5.47c** using just 5% 1,4-dioxane and a final concentration of both alkylating agents of 50 μM. Although the solution containing **5.47c** was slightly turbid, the alkylation of papain seemed to be very efficient and definitely much faster than when using cofactor **5.47a**. Both changes in the structure of the cofactor seemed to have played an important role as not only the cofactor **5.47c** was more soluble than **5.47a** but the labeling compound **5.72** was more reactive than **5.58b** as it can be seen in Figure 5.26, which shows the result of one hour incubation of papain in the presence of **5.72** and **5.58b** using the same final concentration (50 μM) and the same amount of 1,4-dioxane (5%). The residual activity monitored was comparable to the spontaneous hydrolysis of the substrate **5.67** in the absence of the enzyme, also present in Figure 5.26.

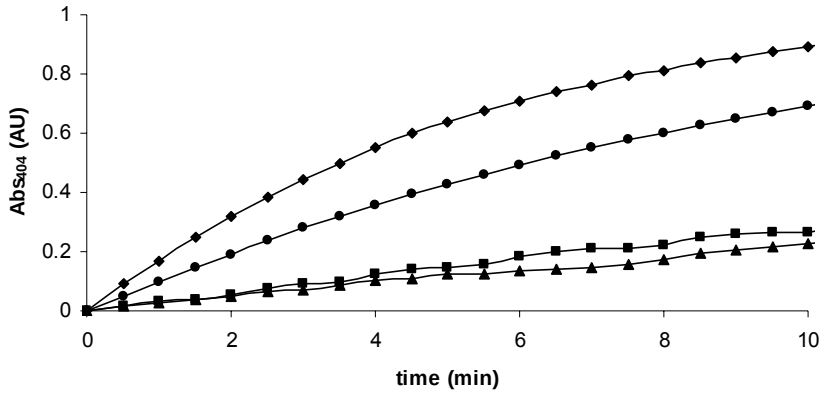


Figure 5.26 *Hydrolysis of **5.67** followed in time by monitoring the release of p-nitrophenolate (**5.68**) by UV-Vis absorption spectroscopy (λ= 404 nm): without papain (■); without affinity label (♦); after 1h incubation with 50 μM **5.72** (▲); after 1h incubation with 50 μM **5.58b** (●).*

Although the lack of activity shown by papain was an important first indication that the alkylation had been successful, it was still indirect evidence. ESI-MS analysis was considered to be probably the most straightforward option to actually identify the modified enzyme.

5.15 ESI-MS analysis of the modified papain adduct PapPhos

Surprisingly, there is still some confusion about the actual mass of papain, the reason for the necessary activation procedure and the state of the Cys-25 in the commercially available enzyme. Although such information does not seem to be

essential whilst using papain simply as a hydrolytic enzyme, it was important for this study and it deserves a small digression.

According to the data sheet provided by Sigma-Aldrich the mass of papain should be 23,406 Da with reference to the sequence reported by Smith and coworkers (1970),[137] which was said to be in agreement with the one reported by Drenth and coworkers determined by crystallographic methods (1968).[70a] However, this mass is wrong as indicated by a more careful literature search. Several other studies on papain reported both the wrong sequence and/or the wrong mass (1965-1971).[138] Kamphuis and coworkers did eventually present a corrected sequence reporting a wrong mass of 23,350 Da (1984).[70b] Just a couple of years later (1986) Cohen and coworkers reported the cloning and sequencing of papain-encoding cDNA which analysis furnished a slightly different sequence of papain.[139] Several sequences are stored in the data banks and they are all available without making any comment on the fact that they are wrong or without removing them.[140] As recently as 2005 a report was published in which papain was shown to have a mass of 23,429 Da by ESI-MS, however, the authors justified their finding by assuming that an atom of Na^+ must be bound to the enzyme again referring to Smith and coworkers (!).[141] The mass of papain used in our study (23,428 Da) is also the most recently reported one by Vernet and coworkers (1989) who prepared a synthetic gene encoding for papain and the sequence obtained was in agreement also with the one connected with the study of Cohen and coworkers.[78]

The mass related to a given sequence can be calculated by using biopolymers calculators or sequencing simulator programs. All these programs provide a protein mass corresponding to a structure in which all the cysteine residues present are in a reduced state. Therefore, as papain possesses 6 cysteine residues beside Cys-25 and they are all involved in disulfide bonds, the mass used as reference during the ESI-MS was 23,422 Da. The use of DTT or other reducing agents for a prolonged time is known to be able to affect the stability of disulfide bonds present in a protein.[137] In consideration of the fact that DTT was used only during the activation and alkylation steps and afterwards removed, this was not considered to be possible.

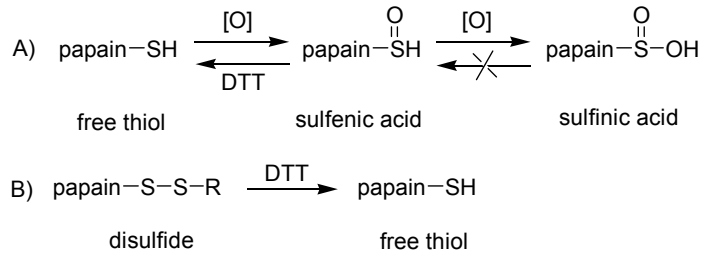
The preparation of the samples for ESI-MS analysis was less straightforward than expected. As previously described, the entire alkylation procedure was performed in phosphate buffer (100 mM, pH 7); however, this buffer is not suitable for ESI-MS as it is non volatile. Therefore, an exchange of buffer to ammonium carbonate was performed using a pre-packed desalting column. Fractions of 1 mL were collected and checked for the presence of protein at 280 nm. Unfortunately, the large excess of cofactor **5.47c** used, which also absorbed at 280 nm, appeared to have been also collected with consequent failure of the ESI-MS analysis. Purification after the alkylation via dialysis was considered a better way of removing the excess of cofactor **5.47c** and the phosphate buffer. For the dialysis to be effective the amount of 1,4-dioxane used during the alkylation step was increased to 10% to help the cofactor **5.47c** to completely solubilize. The solutions containing protein

modified with the linker **5.72**, the complete cofactor **5.47c** and the sample without any treatment were centrifuged five times against pure water with 1% of formic acid. Formic acid was added as it helps the protonation of the protein with consequent better results during the analysis. The use of centrifugal filters allowed also the solutions to first be concentrated and then further diluted to a volume of 200 µL using a solution made up from 70% methanol, 29% water and 1% formic acid and kept at 4 °C overnight. The results of the analysis are presented in Table 5.5.

Table 5.5 *Results of the ESI-MS analysis*

entry	papain	modified with linker **5.72** (**5.76**)	modified with ligand **5.47c** (**5.77**)
expected (Da)	23,422	23,556	24,207
found (Da)	23,454	23,557	24,207

The first analysis was performed on the commercial papain without activation. The observed mass was 23,454 Da. This was a puzzling result as this mass at first could be only assigned to papain in which the free Cys-25 is oxidized to the corresponding sulfinic acid (+32 Da) (Scheme 5.39).



Scheme 5.39 *Thiol, disulfide, sulfenic and sulfinic acids functionalized papain*

Sulfinic and sulfenic acids, which are difficult to detect in normal organic reactions due to their reactivity, have been detected in cysteine-containing enzymes.[142] However, the formation of the sulfinic acid from the sulfenic acid is not reversible, in contrast to the formation of the sulfenic acid from the thiol. Nevertheless, as shown before, upon activation by incubation with DTT papain was active. This gave only few possibilities: 1) papain was commercially available as free thiol oxidized to sulfinic acid during the ESI-MS analysis or during sample preparation due to the absence of DTT;[143] 2) papain was sold as sulfenic acid and therefore unstable; 3) the active Cys-25 was protected as some kind of disulfide which can be reduced (Scheme 5.39); 4) the sulfinic acid could be reduced by DTT. It is reported in the literature that the state of Cys-25 and therefore the role of DTT as reducing agent depends on the way in which papain is extracted and prepared.[124] When directly questioned, Sigma-Aldrich again could not provide any answer. A

disulfide by dimerization of papain itself is also not possible as no reducing agent was used during the analysis.

The second sample analyzed was the one containing papain treated with 3-hydroxy-phenacyl bromide (**5.72**), used as a test reagent for the modification protocol. This adduct **5.76** would also be present in case of hydrolysis of the phosphite. The mass found for **5.76** was 23,557 Da which corresponds to the expected one if an initial mass of 23,422 Da is considered (Table 5.5). The analysis also showed the presence of a small peak with a mass of 23,454 Da, which at this point is still not clear if it corresponds to oxidized papain or to some kind of residual disulfide adduct.

Surprisingly, the analysis of the third sample showed mainly the adduct corresponding to the hydrolyzed cofactor **5.76** (23,557 Da). This alarming degradation of PapPhos (**5.77**) was explained as caused by the presence of 1% formic acid in the sample solution kept at 4°C overnight and final 0.1% during the analysis which might have hydrolyzed the phosphite. A new sample of PapPhos (**5.77**) was prepared and during its purification by dialysis only water was used. Moreover, during the ESI-MS analysis the amount of formic acid was reduced to 0.01%.

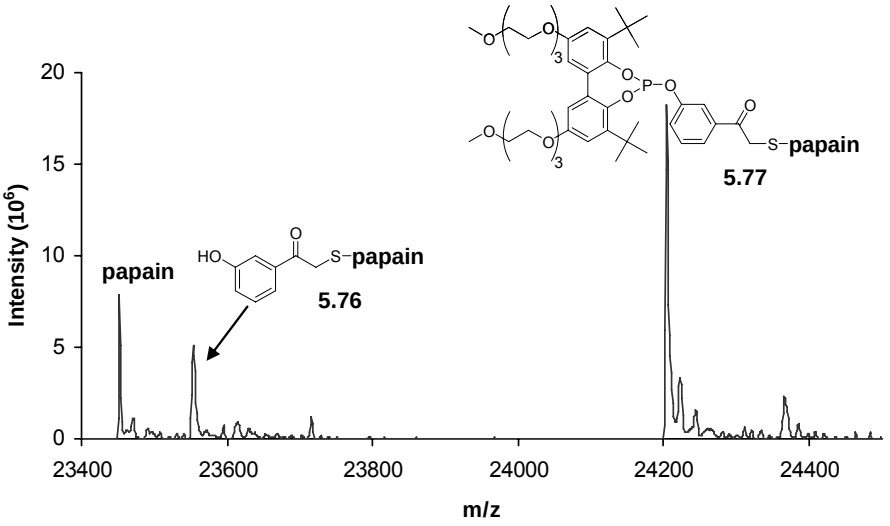


Figure 5.27 *ESI-MS analysis of PapPhos **5.77** resulting from the modification of papain with phosphite **5.47c***

As shown in Figure 5.27, the ESI-MS analysis demonstrated the clear presence of a papain adduct with a mass of 24,207 Da which fitted the expected mass of PapPhos (**5.77**). This confirmed the *successful monoalkylation* of papain with cofactor **5.47c** as expected in consideration of the high reactivity of Cys-25

compared to other residues. The use of 0.01% formic acid significantly reduced the presence of the hydrolysis adduct **5.76**. It was not clear whether the residual presence of **5.76** was still caused by the analysis conditions or if it was already present in the sample. As it was noticed during the analysis of adduct **5.76**, that sample also contained residual not modified papain probably present as sulfinic acid (23,454 Da).

5.16 Digestion of PapPhos (5.77) and tandem mass spectrometry of peptide Asn^{18}-Lys^{39} containing Cys-25.

ESI-MS results confirmed that monoalkylation of papain with cofactor **5.47c** was successfully achieved. The lack of native hydrolytic activity and the rather fast inhibition strongly suggested that Cys-25 was the residue involved in the chemical modification, also due to its much higher reactivity (*vide supra*). In order to confirm that the modification indeed occurred at Cys-25 and not at a close-by residue with consequent conformational distress, trypsin-mediated digestion was performed on native papain and modified **5.77**.[144]

Due to the preference of trypsin to hydrolyze proteins at the C-terminus of arginine and lysine,[145] papain digestion would provide up to 20 specific peptides, one of which (Asn^{18}-Lys^{39}) contains the cysteine residues Cys-25 and also Cys-22.[146] The in-gel digestion allows isolating the peptide of interest which can be analyzed by mass spectrometry. To avoid analysis complications due to thiol oxidation, cysteine residues need to be protected, for example by reaction with iodoacetamide after reduction of all the disulfide bonds present.

Upon activation and treatment with iodoacetamide, the tryptic digestion of native papain, used as reference, will provide peptide Asn^{18}-Lys^{39} (2270 Da) with an increased mass of 2384 Da due to the carbamidomethylation (2 x 57 Da) of both Cys-22 and Cys-25. In the case of modified papain **5.77** the expected mass of the same peptide Asn^{18}-Lys^{39} should be 3114 Da, as the reaction of the protein with iodoacetamide (57 Da) would follow the incubation with **5.47c** (787 Da). The results are shown in Table 5.6.

Table 5.6 *Digestion of papain with trypsin and analysis of peptide Asn^{18}-Lys^{39}*[a]

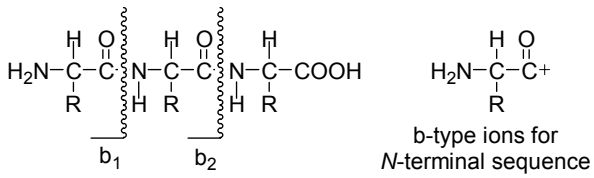
peptide Asn^{18}-Lys^{39}	native papain reacted with iodoacetamide	PapPhos **5.77** reacted with iodoacetamide
expected	2384 Da	3114 Da
found	2384 Da	2463 Da

[a]For the in-gel digestion, the native and modified papain protein bands were electrophoresed in SDS-PAGE gel and then cut out of the gel.

During the mass analysis of peptide Asn^{18}-Lys^{39} of native papain, an ion at 1192.7 *m/z* was found corresponding to the doubly protonated expected ion of peptide Asn^{18}-Lys^{39} (2384 Da). However, during the analysis of the same peptide obtained

from PapPhos **5.77** the expected mass (3114 Da) was not observed. Instead, an ion at 1232.8 *m/z* was found, corresponding to a doubly protonated peptide Asn[18]-Lys[39] with mass 2463 Da. The mass obtained would have been expected from the analysis of **5.76**, therefore after alkylation of papain with linker **5.72** (136 Da). The result clearly showed that the expected peptide of **5.77** had been chemically modified with **5.47c**; however, hydrolysis of the phosphite moiety must have happened during the procedure.

In order to determine which of the cysteine residues, Cys-22 or Cys-25, of peptide [18]NQGS**C**GS**C**WAFSAVVTIEGIIK[39] from **5.77** was modified with what was remaining of the cofactor, the peptide was further fragmented and analyzed by electrospray/tandem mass spectrometry. Fragmentation of peptides during MS/MS analysis provides very characteristic (so called b-type) ions as shown in Scheme 5.40.[147]



Scheme 5.40 *b-Type ions obtained from electrospray / tandem mass spectrometry*

The different level of fragmentation of the parent ion and the fact that each ion is unequivocally characterized by its side chain allow the identification of mass changes for a specific fragment. The expected b-type fragments were calculated for peptide Asn[18]-Lys[39] modified with iodoacetamide at both cysteine residues or with linker **5.72** ligand at either Cys-22 or Cys-25. The results were compared with the fragmentation spectra experimentally obtained (Table 5.7). The product ion scan of the parent ion (1192.8 *m/z*) of the peptide from native papain displayed most of the possible b-fragments including fragments b5 (Cys-22, 548.17 *m/z*) and b8 (Cys-25, 852.23 *m/z*) both showing the iodoacetamide adducts (57 *m/z*). The fragmentation of the parent ion (1232.8 *m/z*) of the modified peptide Asn[18]-Lys[39] from **5.77** showed the expected increase of ion b5 due to carbamidomethylation. A mass increase of 134 m/z starting at b8 was instead attributed to the adduct of what was remaining of **5.47c** at Cys-25. Once more, also this analysis confirmed that the chemical modification using cofactor **5.47c** had been indeed achieved on Cys-25, excluding a possible alkylation of Cys-22.

Table 5.7 *Electrospray/tandem mass spectrometry of peptide Asn[18]-Lys[39] of* ***5.77***

fragments ion $(M+2H)^{2+}$	native papain[a] (*m/z*)	modified at Cys-25[b] (*m/z*)	modified at Cys-22[c] (calculated) (*m/z*)
b1: **N**[18]	115.05	115.05	115.05
b2:N**Q**[19]	243.11	243.11	243.11
b3: NQ**G**[20]	300.13	300.13	300.13
b4: NQG**S**[21]	387.16	387.16	387.16
b5: NQGS**C**[22]	**548.17** (+57)	**548.17** (+57)	**625.17** (+134**)**
b6: NQGSC**G**[23]	605.19	605.19	683.19
b7: NQGSCG**S**[24]	692.23	692.23	770.23
b8: NQGSCGS**C**[25]	**852.23** (+57)	**930.23** (+134)	**930.23** (+57)
b9: NQGSCGSC**W**[26]	1038.31	1115.31	1115.31
b10: NQGSCGSCW**A**[27]	1110.35	1186.35	1186.35
b11: NQGSCGSCWA**F**[28]	1255.42	1333.42	1333.42

[a]Fragment masses found for native papain modified with iodoacetamide at both Cys-22 and Cys-25. [b]Fragment masses found for peptide derived from **5.77**. [c]Fragment masses calculated for modification with **5.47c** at Cys-22.

5.17 Kinetic parameters for the alkylation process

The rate of the irreversible inhibition of papain was determined by incubation of the enzyme for 45 min. in the presence of different concentrations of **5.47c**. Samples of these solutions were tested for residual hydrolytic activity using Z-Gly-*p*-NPE (**5.67**) as substrate at regular intervals.

Inhibition reactions are generally expressed in agreement with the Michaelis-Menten mechanism (Scheme 5.16) which involves the initial formation of a reversible bound enzyme-inhibitor complex followed by covalent modification, in this case irreversible. The kinetic data were processed to fit a minimal kinetic scheme in agreement with an expected irreversible alkylation as depicted in Scheme 5.41:[148]

$$E + I \underset{}{\overset{K_I}{\rightleftharpoons}} E \bullet I \xrightarrow{k_i} E\text{-}I$$

$E^0 = [E] + [E \bullet I] + E\text{-}I$ $\quad$ E^0 = initial enzyme concentration

E-I = alkylated enzyme

$\varepsilon = [E] + [E \bullet I]$ $\quad$ $[E \bullet I]$ = enzyme-inhibitor complex

Scheme 5.41 *Inhibition process treated according to a minimal kinetic scheme*

According to Scheme 5.41, K_I is the apparent dissociation constant of the enzyme-inhibitor complex and k_i is the first-order rate constant of inactivation following the formation of the assumed enzyme-inhibitor complex. Therefore, these constants are expressed as:

$$\frac{[I]\cdot[E]}{[E\cdot I]} = K_I \text{ and } -\frac{d[\varepsilon]}{dt} = k_i[E\cdot I]$$

Equation Error! No text of specified style in document..**1** *Definition of K_I and k_i according to Scheme 5.41*

The amount of initial active enzyme (E^0) and residual active enzyme (ε) can be expressed in terms of residual hydrolytic activity (A_0 and A, respectively). For [I] >> E^0 the observed rate constant (defined as k_{app}) and the solution of the equation become:

A) $Ln\frac{A}{A_0} = -\frac{k_i\cdot t}{1+K_I/[I]} = k_{app}\cdot t$; with B) $\frac{1}{k_{app}} = \frac{1}{k_i} + \frac{K_I}{k_i}\cdot\frac{1}{[I]}$ and C) $k_2 = \frac{k_i}{K_I}$

Equation Error! No text of specified style in document..**2** *Definition of K_I and k_i as function of the hydrolytic activity, providing the apparent second order rate constant k_2*

The relative A/A_0 values listed in Table 5.8 were obtained from the experimental residual hydrolytic activity of papain (0.12 μM) at different moments of the incubation with different concentrations of **5.47c**.

Table 5.8 *Values of relative hydrolytic activity (A/A_0) observed during the incubation time for each concentration of **5.47c***

time (min)	A/A_0 (1.16 μM)	A/A_0 (2.31 μM)	A/A_0 (3.47 μM)	A/A_0 (4.62 μM)
0	1	1	1	1
15	0.377167	0.317993	0.227045	0.227314
30	0.217293	0.143941	0.077458	0.07003
45	0.137463	0.065612	0.056617	0.033704

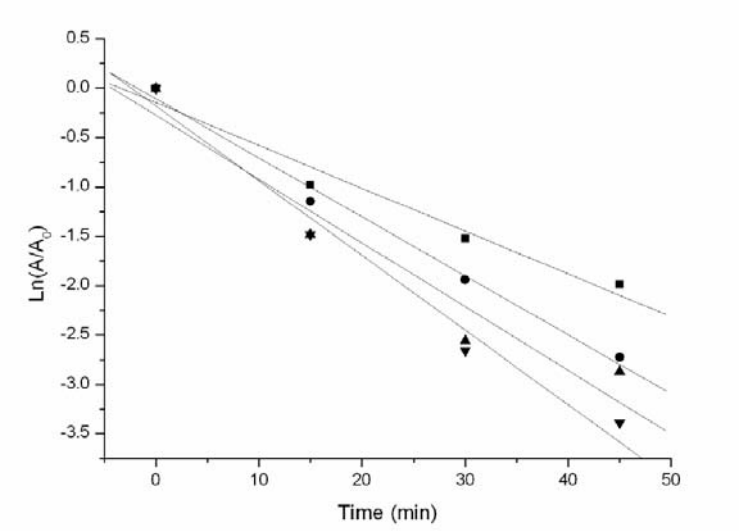


Figure 5.28 *Time dependence of the inactivation-alkylation of papain (0.12 μM) at different concentrations of* ***5.47c*** *plotted as Ln(A/A$_0$) vs. incubation time. Concentrations of* ***5.47c*** *used: 1.2 μM (■), 2.3 μM(●), 3.5 μM(▲), 4.6 μM(▼).*

Figure 5.28 shows the interpolation of the data obtained from the hydrolytic activity of papain with different amounts of **5.47c** as function of the incubation time, which provided a set of k_{app} according to Equation 5.2A (Table 5.9).

Table 5.9 *Values of k_{app} determined for each concentration of* ***5.47c***

5.47c (mM)	k_{app} (min^{-1})
1.16	0.0434
2.31	0.0598
3.47	0.0646
4.62	0.0757

The Kitz-Wilson plot of this values ($1/k_{app}$) as function of the concentration of **5.47c** (1/[I]) is shown in Figure 5.29.

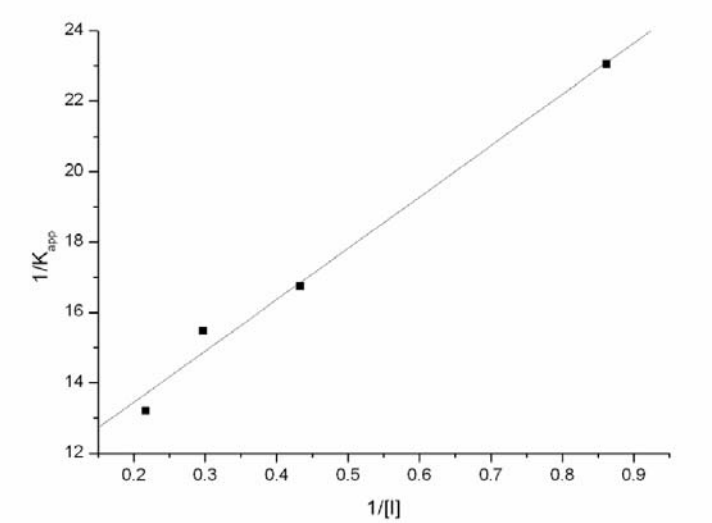


Figure 5.29 *Kitz-Wilson plot of $1/k_{app}$ vs. 1/[I]*

The interpolation of the data allowed to calculate K_I and k_i and consequently k_2 according to Equations 5.2B-C and the results are listed in Table 5.10.

Table 5.10 *Kinetic parameters for the alkylation of papain by **5.47c***

	k_i (min^{-1})	K_I (μM)	$k_i/K_I = k_2$ ($M^{-1}s^{-1}$)
5.47c	0.095	1.385	1150

The apparent second-order rate constant k_2 is a value with which inhibitors are often compared in the literature and can be defined as a measure of the efficiency of the inhibition. For example, k_2 for chloroketone TLCK is 750 $M^{-1}s^{-1}$ and k_2 for the natural inhibitor (*L*) E-64 is 638,000 $M^{-1}s^{-1}$.[65]

5.18 Scaling up the preparation of PapPhos (5.77)

It was first necessary to scale up the amount of protein that could be obtained from the alkylation step, in order to be able to perform any kind of catalysis using **5.77** as ligand for a suitable Rh(I) precursor.

5.18.1 Alkylation protocol

Therefore a new protocol had to be developed. As shown in Table 5.11, **5.47c** was directly added to the activation solution A instead of performing the alkylation step in the more diluted solution B (Table 5.3 and Table 5.4). However, to reach this higher concentration of protein it was necessary to add **5.47c** with a higher final

concentration and the cofactor started to be not completely soluble, as a turbid solution was obtained.

Table 5.11 *New protocol for the scaled up alkylation step*

	volume (mL)	final concentration
P-buffer (100 mM, pH 7)	9.8	100 mM
papain (1.1 mM)	0.1	9.2 µM
DTT (0.1 M)	0.1	10 mM
5.47c (2mM, 1,4-dioxane)	2.0	0.33 mM

Due to the much higher concentration of enzyme, a dilution solution B was prepared to obtain the right concentration of enzyme for the activity test (solution C). Therefore, 50 µL of solution A were diluted to 1 mL (solution B). As before, 90 µL of solution B were added to 890 µL of phosphate buffer (solution C), the solution was placed in a UV cuvette and the substrate **5.67** (20 µL) was added to it as last.

The alkylation step performed according to this new protocol was slower (2-3 h) than the previous one and not always complete. It was not clear whether this was caused by the turbidity of the solution or the lower cofactor **5.47c** to papain ratio (36:1, compared to the previous 91:1). It was tried to dilute the buffer to 50 mM, but this did not improve the results. When 5 mL of cofactor **5.47c** were used, increasing the ratio back to 91:1, no real improvement was noticed. Moreover, the higher amount of final solution (15 mL) became problematic for the following work-up. Not even maintaining the ratio **5.47c** to papain at 36:1 but using more 1,4-dioxane (final 5 mL) helped. Finally a good compromise was found by adding the cofactor **5.47c** in six portions of 0.5 mL (final 3 mL) every 30 minutes.

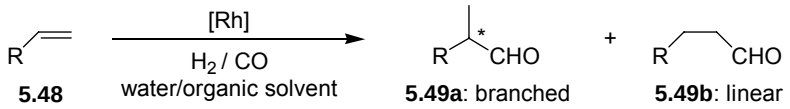
5.18.2 Purification of PapPhos 5.77

As previously described, whilst preparing the samples for the ESI-MS (page 215) it was established that a good way of removing the excess of ligand and concentrating the solutions was dialysis by ultracentrifugation. The centrifugal filters initially used had a 10K cut-off, which means that any molecule smaller than 10,000 NMWL (nominal molecular weight limit) is removed. However, the filters previously used allowed the centrifugation of only 2.5 mL at the time, making the work-up an extremely long process. Other filters adopted (Amicon® Ultra-15) still had a 10K cut-off but an improved capacity of 12 mL whilst using a fixed angle rotor. The presence of a vertical membrane allowed also faster concentration during the centrifugation (from 13 mL to 150 µL in 20 min.). Moreover, the filter seemed to better tolerate the presence of 1,4-dioxane. The solutions at the end of the alkylation step were rather turbid and even dialysis against pure water or in the presence of 50% organic solvent (MeOH, EtOH) did not help eliminating the excess of **5.47c**. As a solution, a different kind of filters was used before the

centrifugation (Minisart SRP 15, 0.45 µm). These filters for syringes allowed to retain the precipitate and clear solutions of **5.77** were obtained, which could be dialyzed against distilled water (3 × 15 mL) and concentrated (final 150 µL) as before. With this improved and reliable purification step in hand, it was decided to test the efficiency of PapPhos in catalysis using Rh(I) precursors. To ensure a maximum degree of reproducibility, adduct **5.77** was always prepared the same day at which the catalysis experiments were performed.

5.19 Rh-catalyzed hydroformylation in aqueous media

Among the possible Rh-catalyzed reactions,[149] hydroformylation (Scheme 5.42) seemed to be an interesting transformation to challenge the newly obtained modified papain **5.77** with, as it cannot be performed by existing enzymes.[8]



Scheme 5.42 *Generic hydroformylation scheme*

This organometallic transformation has been previously performed in aqueous media, which is an important requirement.[150] Biphasic conditions are often inevitable as the alkenes **5.48** used as starting material are generally not water-miscible liquids, therefore, they can be used also as cosolvent or in combination with another organic solvent as, for example, toluene.

Hydroformylation is an important industrial transformation. This means that particular interest has been devoted to solve problems such as separation of the product from the catalyst and catalyst recycling. Performing the reaction in a biphasic media appears to be one possible solution to these issues[151] and the continuous biphasic Rh-catalyzed process developed by Rhône Poulenc and Ruhrchemie AG for the hydroformylation of propene is a successful example.[152] The sulfonated triphenylphosphine **5.78** used is probably still the most well-known water soluble phosphorus based ligand (Figure 5.30). Biphasic hydroformylation reactions are generally performed using temperatures of 40-80 °C and under pressures of 50-80 bar of syngas. Cosolvents often used are toluene, n-hexane, n-heptane and cyclohexane.

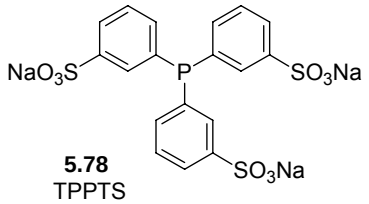
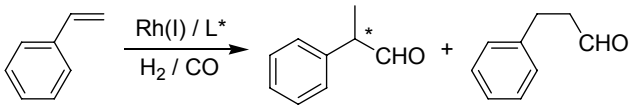


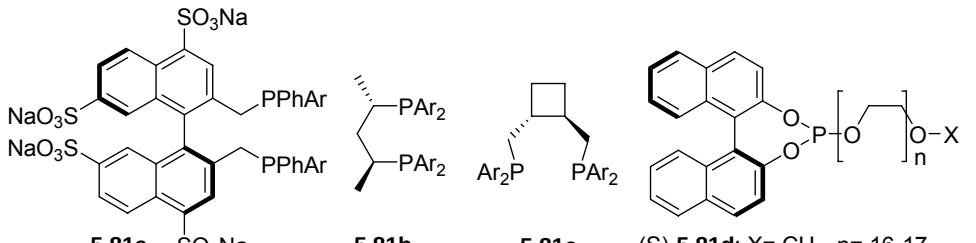
Figure 5.30 *TPPTS (**5.78**), a successful ligand in biphasic hydroformylation*

However, very few chiral water soluble ligands have been used in Rh-catalyzed hydroformylation.[153] Some of the results obtained in the hydroformylation of styrene are depicted in Scheme 5.43.



5.79 **5.80a** **5.80b**

Rh(acac)$(CO)_2$-**5.81a**: 92% conv., **5.80a** 95%, 18% ee (toluene, MeOH, H_2O)
[Rh(μ-OMe)(cod)]-**25.81b**: 67% conv., **5.80a** 76%, 17% ee (MeOH, H_2O)
[Rh(μ-OMe)(cod)]-**5.81c**: 4% conv., **5.80a** 90%, 14% ee (MeOH, H_2O)
$Rh(COD)_2BF_4$-**5.81d**: 71% conv., **5.80a** 76%, 17% ee (H_2O)
$Rh(COD)_2BF_4$-**5.81e**: 99% conv., **5.80a** 83%, 25% ee (H_2O)



5.81a (*S*)-BINAS-6 **5.81b** (*S*,*S*)-BDPPTS **5.81c** (*R*,*R*)-CBDTS (S)-**5.81d**: X= CH_3, n= 16-17 (S,S)-**5.81e**: X= PO_2Binol, n= 4-5

Ar= C_6H_4-3-SO_3Na

Scheme 5.43 *Rh-catalyzed hydroformylation of styrene **5.79** using chiral water soluble phosphorus ligands **5.81a-e***

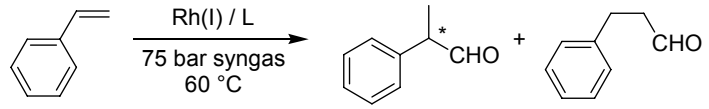
The first use of sulfonated BINOL based bidentate phosphorus ligand **5.81a**[154a] was followed by the report of two examples of chiral alkyl sulfonated diarylphosphines.[154b] Polyether-phosphite ligands **5.81d-e** are instead examples of interesting water-soluble non-ionic monodentate and bidentate phosphite ligands used in thermo-regulated phase transfer conditions.[154c] In all cases, the enantioselectivities obtained for the branched product **5.80a** were rather low and this seems to be a general feature of hydroformylation reactions performed in aqueous media.

5.19.1 Rh-catalyzed hydroformylation reactions using Pap-Phos 5.77 as chiral ligand

The metal precursor Rh(acac)$(CO)_2$ was usually added in considerable excess as a solid to an aqueous solution of **5.77**. The heterogeneous solution was stirred for 30 min. and the excess Rh(I) was filtered away using syringe filters (0.45 μm). A yellowish solution was obtained. The expectation was that only the Rh(I) solubilized by the presence of the protein would remain in solution.

Subsequently, styrene (**5.79**) and the required cosolvent were added and the reaction was performed overnight. Results and reaction conditions are depicted in Table 5.12.

Table 5.12 *Biphasic hydroformylation of styrene (**5.79**) using H_2O / toluene*[a]



5.79 **5.80a** **5.80b**

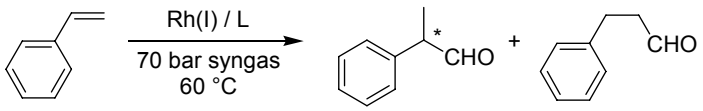
entry	ligand[b]	H_2O : toluene (mL)	conv. (%)	**5.80a** (%)
1	**5.77**	2:2	100	92
2	papain[c]	2:2	100	85
3	-	2:2	100	90
4	**5.77**	3:1	78	92
5	**5.77**	3:0	8	89

[a]Reactions performed in 4 mL of solvent with 2.17 mmol of substrate (250 µL) and 0.11 µmol protein for 16 h. Conversions determined by ^{1}H NMR. Enantioselectivity was determined by HPLC after reduction of the aldehyde to the corresponding alcohol when **5.77** was used. [b]An average of 120 equiv. of $Rh(acac)(CO)_2$ were used. All the solutions were filtered before the addition of toluene and styrene, unless otherwise stated. [c]In this case the excess of Rh(I) was not removed by filtration, Rh(I) to protein ratio 74:1 and a substrate to Rh(I) ratio of 293:1.

The reaction seemed at first to be very efficient (entry 1) as full conversion was obtained with a substrate to protein molar ratio of ≈20,000:1.[155] However, the same result with just slightly lower regioselectivity (85%) was obtained when only papain was used (entry 2). It is possible that the reaction is catalyzed by Rh(I) non specifically bound to the protein structure, although in this case the excess of not solubilized rhodium was not removed. However, when no protein was used full conversion was still obtained (entry 3) pointing at the fact that the reaction could also have been catalyzed by $RhH(CO)_4$ known to be an efficient catalyst. Moreover, the presence of toluene (2:2) as cosolvent could have helped the extraction of the metal from the aqueous layer to the organic one, where the reaction was actually taking place. This possibility was confirmed by a progressive reduction of activity observed when using **5.77** as ligand in the presence of a reduced amount of toluene (entry 4) or without any cosolvent (entry 5). In all cases using papain or **5.77**, product **5.80a** was obtained as a racemic mixture.

It was decided to change the cosolvent as it had been reported that the use of n-heptane helps reducing the leaching of rhodium from the aqueous layer.[156] The results are shown in Table 5.13.

Table 5.13 *Biphasic hydroformylation of styrene (**5.79**) using H_2O / n-heptane*[a]



5.79 **5.80a** **5.80b**

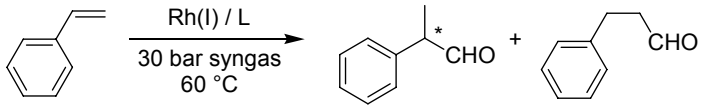
entry	ligand[b]	H_2O : heptane (mL)	conv. (%)	**5.80a** (%)
1	**5.77**	2:2	100	79
2	papain	2:2	100	80
3	-	2:2	80	79
4	**5.77**	3:1	98	79
5	**5.77**	3:0	59	76

[a]Reactions performed in 4 mL of solvent with 2.17 mmol of substrate (250 µL) and 0.11 µmol protein for 16 h. Conversions determined by ^{1}H NMR. Enantioselectivity was determined by HPLC after reduction of the aldehyde to the corresponding alcohol when **5.77** was used. [b]An average of 29 equiv. of $Rh(acac)(CO)_2$ were used. All the solutions were filtered before the addition of n-heptane and styrene.

At the end of the reaction the organic layer was colorless, contrary to the yellow toluene layers that were previously observed and the regioselectivity decreased considerably. Again full conversion was obtained using both **5.77** and papain (entries 1 and 2) and an equal amount of solvents. High but not full conversion was achieved also without the presence of the protein (entry 3). Surprisingly, in this case reducing the amount of cosolvent (entries 4 and 5) resulted in a higher conversion to **5.80a** than previously observed (Table 5.12, entries 4 and 5). These results gave the idea that the metal center might have been more active in the aqueous layer, however the result of entry 3 did not allow to assume that this was due to the presence of the protein. As once more no enantioselectivity was detected for **5.80a** it was also not possible to say if any specific binding was present between the metal center and the phosphite group.

In order to suppress the reaction catalyzed by rhodium not bound to the enzyme and to understand if the phosphite had any influence in the catalysis, another set of experiments was performed in which the syngas pressure was reduced to 30 bar (Table 5.14).

Table 5.14 *Biphasic hydroformylation of styrene (**5.79**) using H_2O / n-heptane*[a]



5.79 **5.80a** **5.80b**

entry	ligand[b]	H_2O : heptane (mL)	conv. (%)	**5.80a** (%)
1	**5.77**	2:2	2.8	64
2	**5.77**	2:2[c]	4.3	64
3	papain	2:2	1.8	66
4	-	2:2	1.4	63

[a]Reactions performed in 4 mL of solvent with 2.17 mmol of substrate (250 µL), 0.11 µmol protein for 16 h, unless otherwise stated. Conversions determined by ^{1}H NMR. Enantioselectivity checked by HPLC after reduction of the aldehyde to alcohol when **5.77** was used. [b]An average of 56 equiv. of Rh(acac)$(CO)_2$ were used. All the solutions were filtered before the addition of n-heptane and styrene. [c]A buffered aqueous solution was used (P-buffer 50 mM, pH 7).

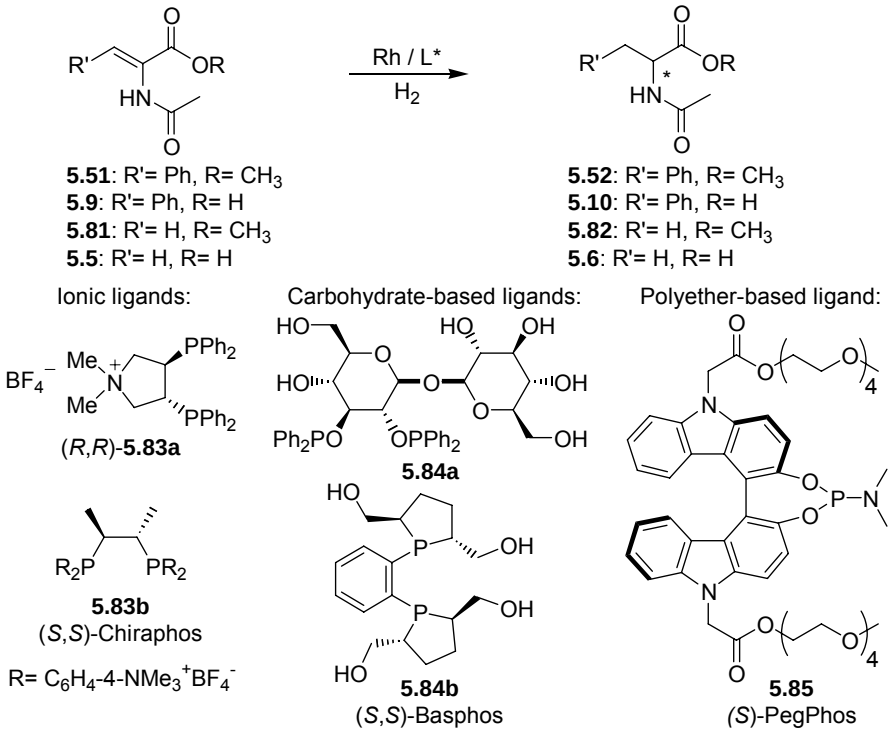
As expected, at this lower pressure, the conversion decreased drastically. However, **5.80a** was still obtained as a racemic mixture with a lower regioselectivity. A higher conversion was obtained when using a buffered aqueous solution instead of water (entry 2). This might have increased the stability of the protein.[157] Interestingly, slightly lower conversions were obtained without **5.77** or in the presence of native papain (entries 3 and 4).

Although the substrate to protein ratio was very high (≈20,000:1) there was no clear evidence that the reaction was indeed performed by rhodium bound to the protein and even less evidence that it was bound to the phosphite group as no enantioselectivity could be induced in all cases. Furthermore, until now biphasic hydroformylation reactions using chiral ligands far more defined than **5.77** provided very low enantioselectivities (Scheme 5.43, 14-25% ee). There was also little information about how the amount of Rh(I) was actually dissolved in solution as even when a lower amount was used (Table 5.13, 19-39 equiv.) all the solutions needed to be filtered. The high temperature and pressure of syngas applied might also cause instability of the protein, although it has been reported that native papain is active and therefore stable at temperatures up to 80 °C.[66]

Biphasic hydroformylation has been defined as a complicated gas-liquid-liquid reaction and despite the number of studies conducted on the various parameters involved, many contradictions are still present in the literature.[158] Because of these reasons, it was decided that hydroformylation reactions were probably not a suitable choice, as a lot of parameters need to be established and understood for this catalytic reaction to be efficient and reliable in aqueous solutions.

5.20 Rh-catalyzed hydrogenation in aqueous media

As discussed in previous chapters, the Rh-catalyzed asymmetric hydrogenation reaction is a very well established transformation. The reaction can be efficiently performed with benchmark substrates using a large variety of both bidentate and monodentate chiral phosphorus based ligands. The advantages of using water as reaction medium are essentially the same as described for hydroformylation reactions. Joó and coworkers were the first to report the use of sulfonated phosphines as ligands in Ru-catalyzed hydrogenation.[159] Subsequently, a number of water-soluble chiral and achiral phosphorus-based ligands have been reported.[160] Hydrophilic groups are generally sulfonate, phosphonate, carboxylate, hydroxy, ammonium, guanidinium, amine and polyether moieties.[161] However, rarely good enantioselectivities were obtained in Rh-catalyzed asymmetric hydrogenation reactions. Some of the most relevant results reported in the hydrogenation of common dehydroamino acids and their methyl esters (Scheme 5.44) are shown in Table 5.15.



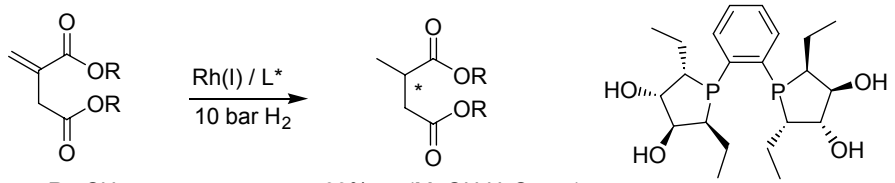
Scheme 5.44 *Rh-catalyzed hydrogenation of α-dehydroamino acids and their methyl esters, using chiral water soluble phosphorus ligands*

Table 5.15 *Rh-catalyzed asymmetric hydrogenation of prochiral substrates in aqueous media using water-soluble ligands* ***5.83-5.85***

entry	ligand	substrate	solvent	ee (%)
1	**5.83a**	**5.9**	H_2O	90
2	**5.83b**	**5.9**	H_2O	94
3	**5.84a**	**5.51**	H_2O:MeOH:EtOAc (6:4:10)	98
4	**5.84a**	**5.51**	H_2O +10% SDS	>99
5	**5.84a**	**5.9**	H_2O:MeOH:EtOAc (6:4:10)	96
6	**5.84b**	**5.81**	H_2O	94
7	**5.84b**	**5.5**	H_2O	>99
8	**5.85**	**5.5**	H_2O:MeOH (1:1)	89
9	**5.85**	**5.5**	H_2O +10% SDS	89

Quaternary ammonium diphosphine ligands **5.83a** and **5.83b** belong to the category of ionic ligands. Their preparation was considered a valuable alternative to sulfonation and their application in asymmetric hydrogenation for example of (*Z*)-2-(acetamido)cinnamic acid (**5.9**) provided good enantioselectivities (entries 1 and 2).[162] Instead, both RajanBabu and Uemura and their coworkers proposed water soluble diphosphinites derived from α,α-trehalose;[163] however, the results obtained using their Rh(I)-complexes in the asymmetric hydrogenation on the same substrates depicted in Scheme 5.44 were rather modest (49-76% ee).[164] In the same paper, Uemura and coworkers reported also the use of the β,β-trehalose derived diphosphinite **5.84a** that proved to be a very efficient ligand in the asymmetric hydrogenation of **5.9** and **5.51** (entries 3-5). Even more interest was directed towards carbohydrate based ligands with the appearance (in the same year) of the D-mannitol derived DuPhos analogue **5.84b** introduced by Börner and coworkers. The Rh(I)-**5.84b** complex provided very good enantioselectivities in the hydrogenation of 2-(acetamido)acrylic acid and its methyl ester (**5.5** and **5.81**, entries 6 and 7). Very recently, a water soluble monodentate phosphoramidite **5.85** has also been presented by our research group in collaboration with the group of Hiemstra and Van Maarseveen.[165] Water solubility was achieved using polyether groups and 89% ee was reached in the hydrogenation of **5.5** using the corresponding Rh(I)-complex (entries 8 and 9). The beneficial influence of the use of surfactants in hydrogenation reactions performed in aqueous media has been frequently reported.[166] For example, the use of 10% SDS eliminated the necessity of cosolvents using both diphosphinite **5.84a** and phosphoramidite **5.85** (entries 4 and 9). Moreover, Uemura and coworkers reported also a considerable increase in reactivity and enantioselectivity.[167]

Zhang and coworkers reported the asymmetric hydrogenation in aqueous media of itaconic acid derivatives **5.7** and **5.86** using chiral ligand **5.84c**, another water-soluble analogue of DuPhos derived from D-mannitol.[168] The excellent enantioselectivities obtained are depicted in Scheme 5.45.



5.86: R= CH_3
5.7: R= H

5.87: >99% ee (MeOH:H_2O, 5:5)
5.8: >99% ee (MeOH:H_2O, 3:97)

Scheme 5.45 *Rh-catalyzed hydrogenation of **5.7** and **5.86** using chiral water soluble carbohydrate-based phosphorus ligand **5.84c***

This overview shows that Rh(I)-catalyzed asymmetric hydrogenation in aqueous media is a much more developed field than the corresponding hydroformylation (Scheme 5.43). The good results obtained by different groups seemed to ensure a better control over the reaction parameters which would help to more easily extrapolate the influence of PapPhos **5.77** from the results. Moreover, milder conditions are used, as the reaction can be performed at room temperature and using relatively low hydrogen pressure.

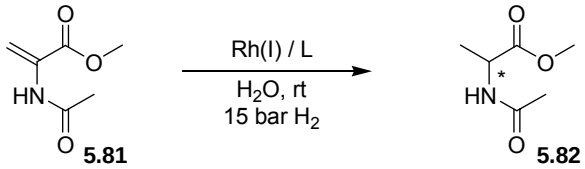
5.20.1 Rh-catalyzed hydrogenation reactions using PapPhos 5.77 as chiral ligand

Methyl 2-(acetamido)acrylate **5.81** was chosen as test substrate due to its good water solubility which provided also easier work up and subsequent analysis. Due to the better solubility of the precursor in water, the removal of the excess $Rh(COD)_2BF_4$ after the complexation was attempted by extraction with toluene. The results of the preliminary attempts are shown in Table 5.16.

Very excitingly, full conversion and 5% ee were obtained in the hydrogenation of **5.81** (entry 1).[169] The hybrid catalyst **5.88** was prepared by the complexation of **5.77** with $Rh(COD)_2BF_4$ added as a stock solution in toluene (15 equivalents). At the end of the complexation time (30 min.) the toluene was removed and the aqueous layer extracted with 1 mL of toluene. The aqueous solution obtained had a yellowish color and a milky appearance. However, the reaction could not be fully reproduced (entry 2) and the control experiment provided by using papain as ligand (*vide infra*) afforded **5.82** in 99% conversion. In another set of experiments the extraction with toluene was performed twice on both **5.88** and Rh(I)-papain. In this case, **5.88** lost almost all the activity but almost full conversion was still obtained for the control experiment (entries 4 and 5). In all cases, turbid gray solutions were obtained after catalysis caused by the presence of rhodium black.

When only 1 equivalent of rhodium precursor was added (from a stock solution in toluene) no conversion was obtained.

Table 5.16 *Rh-catalyzed hydrogenation of methyl 2-(acetamido)acrylate* ***5.81*** *using PapPhos* ***5.77*** *as chiral ligand after extraction with toluene*[a]



5.81 **5.82**

entry	ligand	extraction[b]	conv. (%)[c]	ee (%)[d]
1	**5.77**	1	100	5 (*S*)
2	**5.77**	1	96	2 (*S*)
3	papain	1	99	-
4	**5.77**	2	4	-
5	papain	2	97	-
6	**5.77**[e]	-	-	-

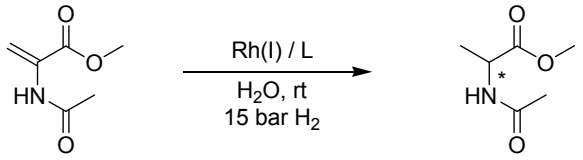
[a]Reactions performed in 2 mL of H_2O for 16 h, using a substrate to protein ratio of 400:1 and a $Rh(COD)_2BF_4$ to protein ratio of 15:1, unless otherwise stated. [b]Number of times the aqueous layer was extracted with toluene. [c]Conversions determined by ^1H NMR and GC. [d]Enantiomeric access determined by chiral GC. [e]Only one equivalent of $Rh(COD)_2BF_4$ was used.

Although an unreliable small degree of enantioselectivity was induced, the reaction did not seem to be particularly reproducible, papain as ligand was as efficient as **5.77** and it was not clear if toluene was indeed a good solvent for the removal of the excess of metal precursor. The use of an equimolar amount of Rh(I) was not enough to obtain an active catalyst.

A few more experiments were performed, in which the removal of the excess of Rh(I) precursor was attempted by extraction with CH_2Cl_2. The metal precursor $Rh(COD)_2BF_4$ was in this case added as a stock solution in CH_2Cl_2 in which it was also more soluble and after 30 min. the organic solvent was removed. The hydrogenation of **5.81** was performed in the presence of **5.88**, Rh(I)-papain and without any ligand. The results are depicted in Table 5.17.

Once more, even after an additional extraction with the organic solvent, almost full conversion was obtained also when using just papain and even without the presence of any ligand (entries 3 and 4). In all cases, after the extraction with CH_2Cl_2 the aqueous solution resulted to be yellow even when only water was used. The product **5.82** obtained was found to be racemic.

Table 5.17 *Rh-catalyzed hydrogenation of methyl 2-(acetamido)acrylate* ***5.81*** *using PapPhos* ***5.77*** *as chiral ligand after extraction with* CH_2Cl_2[a]



5.81 **5.82**

entry	ligand	extraction[b]	conv. (%)[c]
1	**5.77**	1	72
2	**5.77**	2	98
3	papain	2	90
4	-	2	93

[a]Reactions performed in 2 mL of H_2O for 16 h, using a substrate to protein ratio of 400:1 and a $Rh(COD)_2BF_4$ to protein ratio of 15:1, unless otherwise stated. [b]Number of times the aqueous layer was extracted with toluene. [c]Conversions determined by ^1H NMR and GC. [d]Enantiomeric access determined by chiral GC. [e]Only one equivalent of $Rh(COD)_2BF_4$ was used.

Although the Rh(I)-catalyzed hydrogenation seemed to be the adequate catalytic reaction to test the efficiency of **5.77** as chiral ligand, the purity of the catalyst remained an issue. It seemed necessary to have a closer look at the complexation step and alternative ways to purify the complex **5.88**, in order to minimize the amount of residual rhodium precursor and guarantee the stability of the protein and the complex.

5.21 Complexation of PapPhos (5.77) with $Rh(COD)_2BF_4$

The introduction of cofactor **5.47c** in the active site of papain afforded a hybrid protein structure which has lost its original hydrolytic activity and cannot be defined as an enzyme anymore. The preferential and stable complexation of a metal precursor to the newly introduced non-proteinogenic moiety would convert the hybrid protein into a metalloprotein, which could be considered a bio-organometallic catalyst in case reliable catalysis could be performed. The experimental results previously shown, demonstrated that it was not possible to make such a statement just yet, as the catalysis was not reproducible or selective. This can be attributed to a lack of control over the complexation step and the purification of the complex obtained (Figure 5.31). As the rhodium precursor was added to the already modified protein, it is important to understand the entity of the possible interactions between the metal center and the different residues of papain.

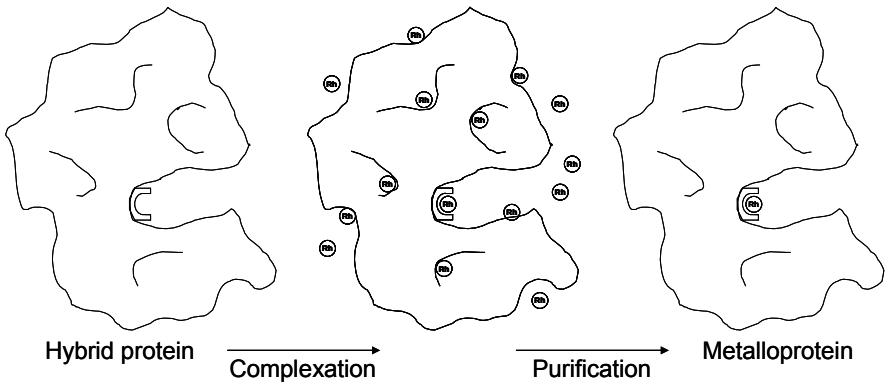


Figure 5.31 *Metal complexation: from hybrid protein to metalloprotein*

It was assumed that the rhodium would preferentially bind to a phosphite rather than an amino acid side chains or the protein backbone. However, due to the excess of catalyst precursor used, unspecific binding may occur, which would lead to the presence of metallic rhodium during the hydrogenation reaction. Thus, hydrogenation with metallic rhodium would dominate the results. It was also shown that the addition of only one equivalent of metal precursor failed to provide any catalytic activity. Only one equivalent was insufficient but the use of an excess of metal precursor required a better purification protocol than what had been used so far.

5.21.1 Papain as a source of binding sites

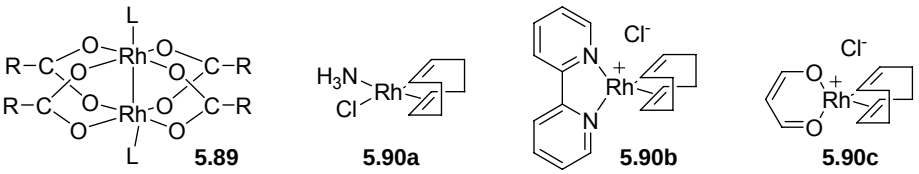
Metalloprotein reactivity is generally tuned by the presence of different metal ions and coordination geometries and 1/3 of all the structurally characterized proteins contain metal-binding sites.[25] The physiologically most relevant metals are in evidence in the periodic table depicted in Figure 5.32.[24]

H																	He
Li	Be											B	C	N	O	F	Ne
Na	**Mg**											Al	Si	P	S	Cl	Ar
K	**Ca**	Sc	Ti	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	**Mo**	Tc	**Ru**	**Rh**	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	**W**	Re	Os	**Ir**	**Pt**	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Unq	Unp	Unh												

Figure 5.32 *The metals of biology (bold text) and transition metals used in antitumoral studies (bold text and cells)*

Rhodium obviously is not among these metals, but an increased interest in finding less toxic organometallic compounds with antitumoral activity has stimulated

studies on metals such as rhodium, ruthenium and iridium.[170] For this reason, different dimeric μ-acetato dimers of Rh(II), monomeric square planar Rh(I) and octahedral Rh(III) complexes have been studied and showed interesting properties,[171] mostly concerning their interactions with DNA (Figure 5.33).[172]



5.89 **5.90a** **5.90b** **5.90c**

Figure 5.33 *Rh(II) carboxylates **5.89** and few of the Rh(I) complexes **5.90** used as alternative cytostatic drugs*

To obtain insight into the molecular modes of action of intravenously administered antitumoral metal complexes, investigations have been conducted on the interaction of Rh(II) carboxylates **5.89** with serum proteins.[173] Human serum albumin (HSA), for example, is the principal protein component of plasma; as such, it binds and transports a wide variety of substances, including metals. Rh(II) carboxylates **5.89** have shown to form relatively stable mono and bis-adducts with a variety of residues of HSA possessing donor atoms such as nitrogen, sulfur, and oxygen.[174] Spectroscopic studies using a variety of amino acids indicated that only histidine, cysteine and methionine form adducts with $Rh_2(OAc)_4$, histidine being the preferred binding site.[175] Tryptophan seemed to provide a weaker binding site. It was also observed that α-amino acids containing free sulfhydryl groups could lead to disruption of the Rh-Rh bond with formation of monomeric chelate species.

In conclusion, according to the literature studies, histidine, methionine, cysteine and to some extent tryptophan were assumed to be suitable residues for metal binding. In consideration of the presence of such residues in papain (Figure 5.34), it was assumed that 8 equivalents of $Rh(COD)_2BF_4$ could complex to the protein.

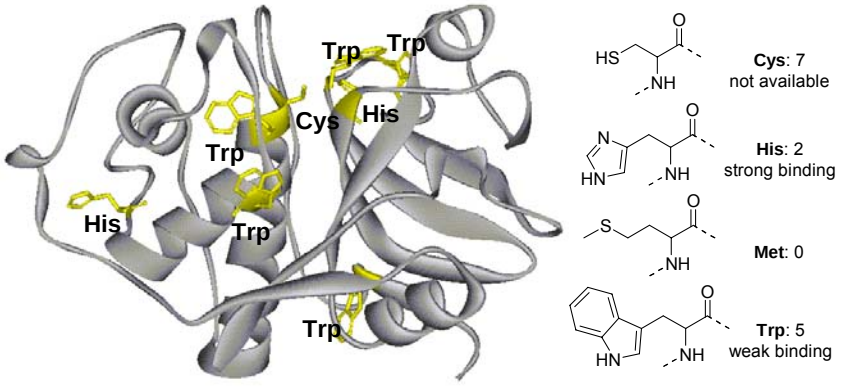


Figure 5.34 *Papain residues as potential metal-binding sites*

None of the cysteine residues were considered available as six of them were involved in disulfides bonds and Cys-25 carried the phosphite group which counts as one binding site. Nevertheless, eight equiv. of Rh(I) could well be an excess as, for example, a couple of the indole residues are buried in the structure and might not be accessible.

5.21.2 Improvements in the purification of Rh(I)-PapPhos complex

Purification of the conjugate **5.88** obtained after the complexation of Pap-Phos **5.77** with $Rh(COD)_2BF_4$ was pursued in a milder and more efficient way using two different prepacked desalting columns, which both follow the principal of size exclusion chromatography. One column was prepacked with Sephadex™ G-25 (HiTrap™) and connected with an MPLC[176] system (AKTA purifier). The second column was prepacked with polyacrylamide Bio-Gel® P-6DG gel (Econo-Pac®) and the purification was performed under atmospheric pressure. The protein was eluted first using both systems, which allowed the separation from excess or weakly bound Rh(I) and an exchange of buffer if necessary. In this respect, both systems efficiently replaced a dialysis step. In both cases, the fractions collected during the purification were checked for the presence of protein by UV at 280 nm. However, the MPLC system had the advantage of having a graphic interface which allowed in real time to monitor the elution at different wavelengths and Figure 5.35 is an example of the UV profile obtained.

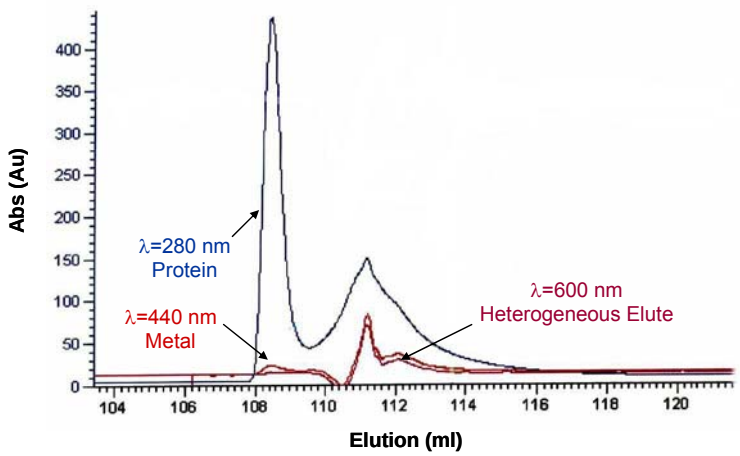


Figure 5.35 *UV profile obtained during MPLC purification of the conjugate **5.88***

It was possible to detect at three different wavelengths: 280 nm identified the protein, 440 nm was related to the presence of metal, 600 nm was related to the presence of heterogeneous elute. During this particular purification the complexation was performed using 15 equivalents of rhodium precursor. More interestingly, the fractions containing protein also showed a band at 440 nm and the same band was overlapping the band at 600 nm, thus confirming that the

purification allowed to remove the excess of metal present in solution. However, there was no illusion that only one equivalent of Rh(I) was bound to the protein and coordinated to the phosphite group and indeed the same purification performed on not modified papain also showed the presence of a band at 440 nm eluting with the protein.

Spectroscopic studies conducted on cationic Rh(I)-diphosphine complexes in methanol reported UV-Vis absorptions between 385 and 442 nm of rather low intensity (ε= 1.6-5.0 10^{-3} M^{-1} cm^{-1})[177] compared to the intensity of the nearby band of papain at 280 nm (ε= 57.5 10^{-3} M^{-1} cm^{-1}). The profile of the elution confirmed the large difference in intensity, as also observed in preliminary UV studies.

5.21.3 ESI-MS analysis of Rh(I)-PapPhos complex 5.88

Once an improved purification method was established, ESI-MS was again used to identify what kind of complex had been obtained and, if possible, how many equivalents of Rh(I) had been incorporated in PapPhos and papain itself. The use of the MPLC system yielded the conjugates in good purity.

During the complexation eight equivalents of $Rh(COD)_2BF_4$ were added from a stock solution in dioxane to a solution in pure water of both freshly prepared PapPhos and papain. The solution was allowed to stir for one hour after which time it was purified with pure water using the MPLC system previously described. The solutions of the purified conjugates were stored overnight at 4 °C. The ESI-MS spectrum obtained for **5.88** is shown in Figure 5.36.

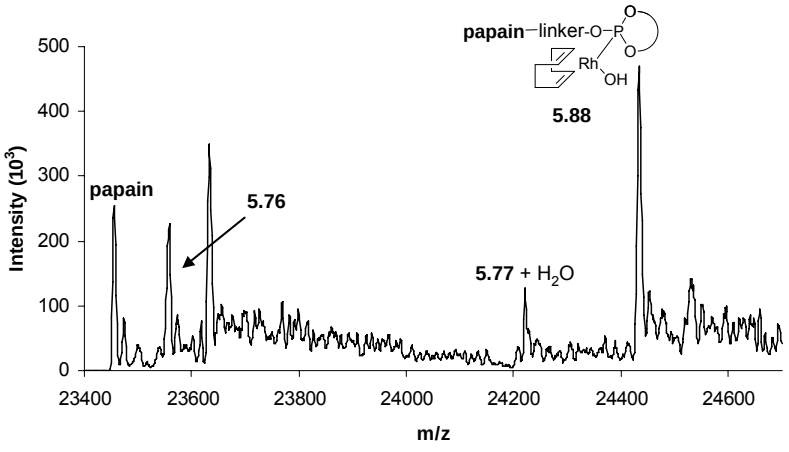


Figure 5.36 *ESI-MS analysis of Rh(I)-PapPhos complex **5.88***

Very excitingly, mass spectral analysis of **5.88** showed the disappearance of the peak at 24,207 Da (**5.77**) and the appearance of a clear and distinct peak at

24,434 Da (Table 5.18) corresponding to the addition of Rh(COD) (211 Da) and possibly a molecule of water. This confirmed the presence of a single rhodium which was assumed to be solely bound to the phosphite group, this being the best ligand for the metal. An additional peak (23634 Da) appears in the ESI-MS, which could be only attributed to an adduct between papain in its reduced state and one unit of Rh(COD). However, this is probably an artifact, as in all the experiments the reduced papain was reacted completely with the cofactor or was oxidized.

Table 5.18 *Results of the ESI-MS analysis of conjugate **5.88***

entry	native papain	**5.77**	**5.77** complexed with $Rh(COD)_2BF_4$
expected (Da)	23,422	24,207	-
found (Da)	23,455	24,207	24,434

The same analysis was performed on native papain and a complex and unclear spectrum was obtained, which is shown in Figure 5.37. None of the peaks could be identified. This does not mean that no adduct between Rh(I) and papain was present, as such complex formation was evident from the UV-Vis absorption at 440 nm detected during the purification. Instead, this result showed that if any adduct was present it was quite labile, as it could not survive the otherwise mild conditions of the analysis.

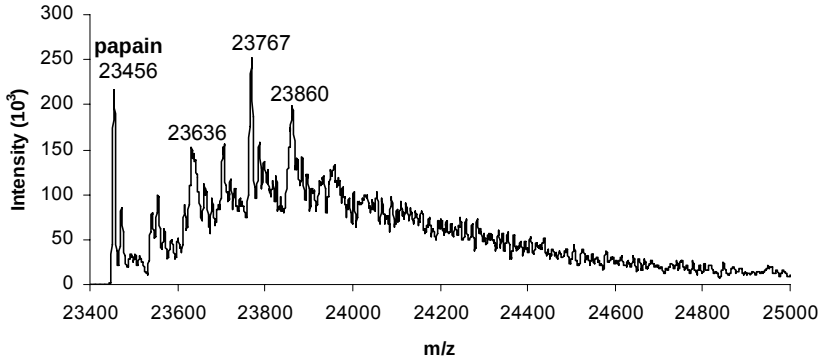


Figure 5.37 *ESI-MS analysis of papain after complexation with $Rh(COD)_2BF_4$*

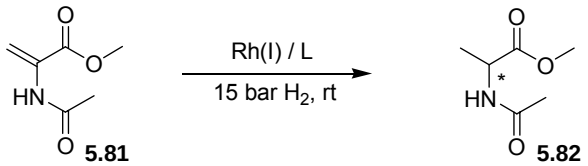
The results of the ESI-MS analysis also proved that a good level of purity had been achieved after purification of the product of the reaction with $Rh(COD)_2BF_4$. The impact of this improved preparation on the activity of complex **5.88** was tested by performing a new set of hydrogenation reactions.

5.22 Hydrogenation of methyl 2-(acetamido)acrylate (5.81) after the new purification procedure of complex 5.88

All the hydrogenation reactions were performed after purification of the complex **5.88** using the MPLC system. Generally, the complex was recovered after purification as a 1.5 mL solution. The substrate **5.81** was added as a stock solution in the same solvent (buffer or H_2O). The experimental results, which also provided new information on how to improve the procedure, are depicted in Table 5.19.

At first it was decided to perform both complexation and hydrogenation reaction in phosphate buffer to see if this would have improved the stability of the protein and would have had an influence on the results. An excess of $Rh(COD)_2BF_4$ was used (15 eq., added as a solid) and the solution was stirred for one hour before purification. The same procedure was adopted also with native papain used as a reference for undesired catalysis caused by random and not specific binding of rhodium to the protein structure.

Table 5.19 *Rh-catalyzed hydrogenation of methyl 2-(acetamido)acrylate **5.81** using PapPhos **5.77** as chiral ligand*[a]



5.81 **5.82**

entry	ligand	Rh(I) eq. (state)	complexation solvent	hydrogenation solvent	conv. (%)[b]
1	**5.77**	15 (solid)	buffer	buffer	79
2	papain	15 (solid)	buffer	buffer	89
3	**5.77**	4 (buffer)	buffer	buffer	-
4	papain	4 (buffer)	buffer	buffer	3
5	**5.77**	8 (1,4-dioxane)	H_2O	H_2O	-
6	papain	8 (1,4-dioxane)	H_2O	H_2O	3

[a]Reactions performed in 2 mL of H_2O or phosphate buffer (25 mM, pH 7) for 16 h, using a substrate to protein ratio of 400:1. [b]Conversions determined by ^1H NMR and GC. Enantiomeric access determined by chiral GC.

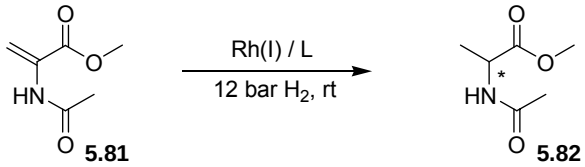
Unfortunately, conversion was detected in both cases (entries 1 and 2). A reason for the undesired activity was attributed to an overlap between the protein eluted and excess metal precursor during the purification step as shown in Figure 5.35. Therefore, less rhodium was used (entry 3 and 4) and for convenience it was

added to the solution containing **5.77** or papain from a stock solution in phosphate buffer. In this case, no excess of metal precursor eluting after both proteins was visible at 440 nm which meant that it was all bound to **5.77** or papain, however, no activity was detected with both systems in the hydrogenation of **5.81**. The purification in this case proceeded efficiently and the lack of activity was attributed to a possible insufficient amount of metal precursor. Moreover, the stock solution of Rh(I) in phosphate buffer was slightly turbid.

Due to the good results obtained during ESI-MS analysis (page 239), the same conditions for the preparation of the complex **5.88** were applied, using eight equivalents of rhodium from a stock solution in 1,4-dioxane and performing the complexation and the hydrogenation of **5.81** in pure H_2O (entries 5 and 6). The combination of H_2O and 1,4-dioxane (10%) during the complexation step afforded a clear yellowish solution. This amount of precursor allowed a good purification between the protein and the excess of Rh(I). However, no activity was detected using both **5.77** and papain as ligands. It was in this case assumed that the purification in the absence of buffer might have compromised the stability of the protein.

As a compromise, it was decided to perform the complexation in H_2O and exchange it to phosphate buffer during the purification of the conjugate **5.88** and the results are shown in Table 5.20.

Table 5.20 *Rh-catalyzed hydrogenation of methyl 2-(acetamido)acrylate **5.81** using PapPhos **5.77** as chiral ligand*[a]



5.81 **5.82**

entry	ligand	conv. (%)[b]
1	**5.77**	100
2	papain	2
3	**5.77**	97
4	papain	2
5	**5.77**[c]	100, **22% ee** (*R*)
6	**5.77**[c]	100

[a]Reactions performed in 2 mL of phosphate buffer (25 mM, pH 7) for 16 h, using a substrate to protein ratio of 400:1 and a $Rh(COD)_2BF_4$ to protein ratio of 8:1, unless otherwise stated. [b]Conversions determined by ^1H NMR and GC. Enantiomeric access analyzed by chiral GC. [c]A substrate to protein ratio of 800:1 was used.

It was pleasing to see that under these conditions full conversion in the hydrogenation of **5.81** was obtained by using **5.88** as catalyst (entry 1 and 3). The reaction showed to be reproducible and negligible activity was observed using papain as control (entries 2 and 4). This result confirmed that the phosphite-bound rhodium was responsible for the observed catalytic activity. Moreover, full conversion under 12 bar H_2 pressure was also obtained when the substrate to catalyst ratio was increased to 800:1 (entry 5 and 6). On one single occasion **5.82** was obtained with 22% enantioselectivity (entry 5), although this result could not be reproduced (entry 6).

By repeating the reaction a number of times, a progressive decrease of activity from 97% (entry 3) to eventually 17% conversion became evident. Eventually, it was discovered that this was related to the continuous use of the same prepacked Sephadex™ G-25 (HiTrap™) column for the purification of the Rh-protein conjugates. This was confirmed by a regain of activity observed when the other desalting column available was used (polyacrylamide Bio-Gel® P-6DG gel). The reason for the degradation of the column over time could not be clearly identified.

5.23 Conclusions and outlook

In conclusion, it has been shown that it is possible to convert a hydrolytic enzyme into a unique fully functional hydrogenation catalyst by attaching a single phosphorus ligand to its active site and treating it with a rhodium precursor. The hydrogenation reaction performed using **5.88** yielded the desired alanine derivative **5.82** with 100% selectivity. The protocol achieved seemed to be reliable and effective, as no activity was detected when using native papain treated with Rh(I) precursor and purified in the same way. The lack of induction of enantioselectivity points to a catalytic system in which the metal site could be too flexible or too far removed from the chiral environment of the enzyme. In order to understand what the new active site might look like some molecular modeling was attempted (Figure 5.38).

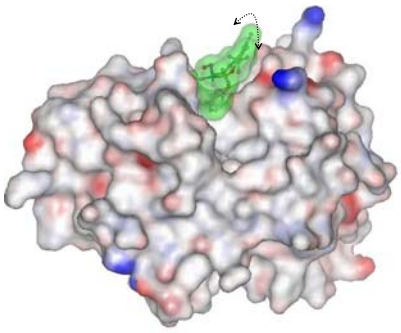


Figure 5.38 *Manual docking of **5.47c** into the active site of papain*

Although programs and algorithms for protein molecular modeling for the minimization of modified structures are available, they are not compatible with the insertion of non-proteogenic structures or organometallic complexes. Therefore, it was only possible to obtain a manual docking of the cofactor **5.47c** anchored to Cys-25 in the active site of papain.

One of the reasons for choosing papain as host for the organometallic catalyst was that its cavity was assumed to be spacious enough to accommodate ligand, metal and substrate. It was also hoped that additional binding sites for Rh(I), if necessary, could be found between the residues in the enzyme pocket, as for example His-159 positioned opposite to Cys-25.[70,71] Although only indicative, Figure 5.38 showed that the cavity might be too large to guarantee enough secondary interactions necessary to ensure a more rigidly bound metal complex. In all cases multiple conformations of the complex could be present and the phosphite moiety would be free enough to flip in the cavity.

The cavity of papain could have facilitated the induction of chirality in different ways: (1) by providing an extra coordination for the unsaturated Rh-monophosphite complex; (2) by forcing the structurally fluxional phosphite backbone in a preferred conformation; (3) by favoring a specific complexation of the substrate to the catalyst due to the bulk of the protein itself. None of these interactions between papain and the catalyst or the substrate could be predicted before hand. Therefore, although disappointing, it is not completely surprising that no induction of enantioselectivity was observed.

The important target achieved was the establishment of a rational and reliable protocol for the attachment of the ligand and the complexation of the metal precursor to the hybrid protein. At this point, the challenge would be to be able to randomly or specifically modify the protein backbone in search for the necessary secondary interactions to guarantee stereocontrol. Of course, this could also be achieved by screening completely different protein structures as scaffold. In this respect, a more stable and conformationally defined complex could also be obtained by double anchoring of the ligand to the protein structure. An interesting approach was recently reported by Lu and coworkers, who showed the beneficial effect of the two points attachment of a salen catalyst into Apo-Mb on the enantioselectivity of the sulfoxide product (Scheme 5.12).[45]

The establishment of a covalent approach allows a wide range of possibilities in terms of choice of protein structure and cofactor design. However, it is at this point not surprising anymore that more examples have been reported using a supramolecular approach (page 174). From a practical point of view, a non-covalent approach requires less protein manipulation and purification, which, besides being time consuming, require extensive preparation and purification protocols and might hamper the stability of the protein itself.

Exciting progresses could be obtained in the field of artificial metalloproteins by modification or novel design of a metal-binding pocket with the right geometry

between the different residues in order to accommodate different metals, as an expansion of the work of Sheldon and coworkers (Scheme 5.14).[50] An interesting but not exploited approach is also the introduction of unnatural α-amino acid derivatives in the backbone of a protein as suggested by Imperiali and Roy (Scheme 5.14).[48] The possibility of engineering a gene that would code for the desired α-amino acid which already bears a metal-binding side chain instead of using solid state synthesis would make this approach even more exciting.

Looking at the preparation of semisynthetic metalloproteins as an expansion of the combinatorial approach for the identification of new efficient catalysts with novel catalytic properties, we should ask ourselves in how much these fascinating but still tailor-design approaches can cope with the fast tempo involved in high throughput experimentation, in particular of low molecular weight catalysts. However, the growing enthusiasm and commitment shown by the scientific community in embracing a truly interdisciplinary mentality might provide for this new field exciting and fast progress in the near future.

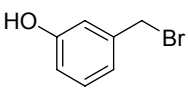
5.24 Experimental section

General remarks

For general remarks, see Chapter 2. For synthetic purposes, solvents were reagent grade, dried and distilled before use following standard procedures. Dioxane was distilled over sodium under nitrogen or purified over neutral alumina and degassed; buffer solutions and water were degassed for 2-3 h prior to use. Papain was obtained from Sigma-Aldrich as a buffered aqueous suspension in 0.05 M sodium acetate (1.1 mM). *N*-*Z*-glycine *p*-nitrophenyl ester was purchased from Bachem. DTT (dithiothreitol) was obtained from Roche. Mass spectra were recorded on an AEI-MS-902 mass spectrometer. UV-Vis measurements were performed on a Hewlett-Packard HP 8453 FT spectrophotometer. Enantiomeric excesses were determined by capillary chiral GC analysis on a HP 6890 gas chromatograph equipped with a flame ionization detector.

The kinetics of papain inhibition with **5.47c** and the digestion of **5.77** were performed by Jianfeng Jin (department of biochemistry). In-gel digestion was performed as previously described in the literature.[144] All the mass analysis (ESI-MS and MS/MS) were performed in collaboration with C. M. Jeronimus-Stratingh and/or H. Permentier (faculty centre for mass spectrometry). Manual docking of **5.47c** in the active site of papain was performed by Marco W. Fraaije.

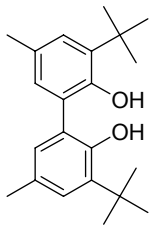
3-Bromomethyl-phenol (5.58b)



Method A.[112] Pyridine (2.6 mL, 32.4 mmol) in dry THF (5 mL) was added to a solution of PBr_3 (10.2 g, 37.7 mmol) also in THF (10 mL) at -5 °C. Finally, a solution of alcohol **5.57b** (13.9 g, 112 mmol) in dry THF (170 mL) was added dropwise. The

reaction mixture became turbid. It was allowed to reach room temperature and stirred overnight. The mixture was then filtered over celite that was washed with dry THF. The solvent was removed at room temperature under reduced pressure and a dark red oil was obtained. Toluene (100 mL) was added and the solution obtained was kept at -20 °C for 2 h and filtered over celite. The light brown solution obtained was stored at 4 °C. Eventually, the solvent was removed and purification by flash column chromatography on silica gel (toluene) afforded product **5.58b** (50%). **Method B**.[114] A solution of PBr_3 (4.4 g, 16.1 mmol) in $CHCl_3$ (18 mL) was added to a suspension of alcohol **5.57b** (4.0 g, 32.2 mmol) in $CHCl_3$ (18 mL) at 0 °C. At the end of the addition a clear solution was obtained which was allowed to reach room temperature and was stirred for additional 2 h. The reaction was stopped by addition of ice (36 g) and separation of the organic layer. The aqueous layer was extracted with $CHCl_3$ (3 x 40 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The product **5.58b** was obtained pure as an oil (98%) and stored at 4 °C, where it solidified. After a couple of months, **5.58b** was purified by flash column chromatography on silica gel (toluene) and recovered with 90% yield.
M.p. 65.5-66.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.21 (t, *J*= 8.0 Hz, 1H), 6.96 (d, *J*= 7.6 Hz, 1H), 6.88 (t, *J*= 2.0 Hz, 1H), 6.77 (dd, *J*= 2.4, 8.0 Hz, 1H), 4.95 (br s, 1H), 4.44 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.6 (s), 139.4 (s), 130.0 (d), 121.4 (d), 115.9 (d), 115.5 (d), 33.1 (t). MS, *m/z* (%): 188 (^{81}Br M^+, 18.5%); HRMS for $C_7H_7O^{81}Br$, calcd: 187.966, found: 187.967.

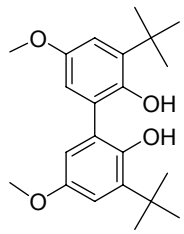
3,3'-Di-tert-butyl-5,5'-dimethyl-biphenyl-2,2'-diol (5.62a)



Method A.[117] Phenol **5.60a** (25.0 g, 152 mmol) was dissolved in dry MeOH (64 mL) under a nitrogen atmosphere and $CuCl_2$ (0.1 g, 0.7 mmol) was added to this solution. TMEDA (250 µL, 1.6 mmol) was added dropwise and the solution turned from yellow to dark green. Vacuum was applied and nitrogen was replaced by pure oxygen. The reaction mixture was stirred at room temperature and it was stopped after 5 d. The work up consisted of addition of water and extraction with $CHCL_3$. The organic layer was washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (toluene) afforded the desired product **5.62a** as an off-white solid (20%). **Method B**.[120] Phenol **5.60a** (5.0 g, 30.4 mmol) and $FeCl_3$ $6H_2O$ (2.0 g, 7.6 mmol) were dissolved in CCl_4 (76 mL). After a solution of *t*-BuOOH (3.0 mL, 5.6 M in n-decane) was added the reaction mixture was heated at 80 °C for 3 h.[178] The solution was washed with 10% aqueous HCl, the organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (toluene) afforded the desired product **5.62a** (69%).
M.p. 98.5-100.0 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.15 (s, 2H), 6.90 (s, 2H), 5.18 (s, 2H), 2.31 (s, 6H), 1.44 (s, 18H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 149.8 (s, 2C), 136.9 (s, 2C), 129.5 (s, 2C), 128.7 (d, 2C), 128.4 (d, 2C), 122.5 (s, 2C), 34.9 (s,

2C), 29.6 (q, 6C), 20.8 (q, 2C). MS, *m/z* (%): 326 (M^+, 97.6%); HRMS for $C_{22}H_{30}O_2$, calcd: 326.225, found: 326.224.

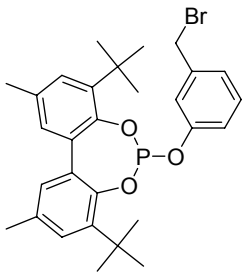
3,3'-Di-tert-butyl-5,5'-dimethoxy-biphenyl-2,2'-diol (5.62b)[179]



A solution of phenol **5.60b** (4.3 g, 24 mmol) in acetone (50 mL) was added to a solution of $K_3[Fe(CN)_6]$ (8.2 g, 25 mmol) in H_2O (80 mL) to yield a yellow/orange mixture. Subsequently, an aqueous solution of NaOH (0.95 g, 23.8 mmol, 10 mL H_2O) was added and the solution turned blue/green. The reaction mixture was stirred for 2 h at room temperature, after which time it was extracted with $CHCl_3$ (200 mL), the organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The desired product **5.62b** was obtained as a pinkish solid after recrystallization from MeOH (60%).

M.p. 224.7-226.0 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.96 (d, *J*= 3.0 Hz, 2H), 6.63 (d, *J*= 3.0 Hz, 2H), 5.02 (s, 2H), 3.78 (s, 6H), 1.43 (s, 18H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 153.2 (s, 2C), 145.9 (s, 2C), 138.9 (s, 2C), 123.1 (s, 2C), 115.3 (d, 2C), 111.7 (d, 2C), 55.7 (q, 2C), 35.2 (s, 2C), 29.5 (q, 6C). MS, *m/z* (%): 358 (M^+, 100%); HRMS for $C_{22}H_{30}O_4$, calcd: 358.214, found: 358.212.

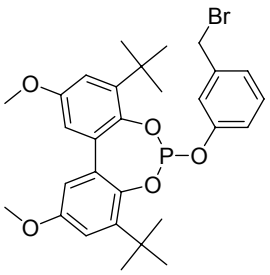
6-(3-Bromomethyl-phenoxy)-4,8-di-tert-butyl-2,10-dimethyl-5,7-dioxa-6-phospha-dibenzo[a,c]cycloheptene (5.47a)



PCl_3 (250 µL, 2.9 mmol) was added dropwise to a solution of NEt_3 (2 mL, 12.4 mmol) in dry toluene (5 mL) at 0 °C. Bisphenol **5.62a** (0.92 g, 2.8 mmol) was dried by repeated addition and removal under reduced pressure of toluene (5 mL, 3 times) and then dissolved in toluene (10 mL) and added dropwise to the reaction mixture at 0 °C. The yellow solution was allowed to reach room temperature and was stirred for 3 h, until the starting material PCl_3 was not visible by ^{31}P-NMR (202 ppm). The reaction mixture was cooled to 0 °C and NEt_3 (0.8 mL, 5.8 mmol) was added. Benzyl bromide **5.58b** (0.53 g, 2.8 mmol) was dried by repeated addition and removal under reduced pressure of toluene (5 mL, 3 times) and then dissolved in toluene (10 mL) and added dropwise to the reaction mixture. Stirring at room temperature was continued for 3 h, after addition of Et_2O (5 mL) the salts were filtered and the solvent was removed under reduced pressure. The desired product **5.47a** (50%) was obtained after purification by flash column chromatography on silica gel as an oil (n-hexane and 0.5% NEt_3) and it was stored at -12 °C.

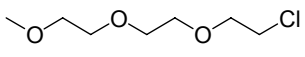
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29-7.22 (m, 3H), 7.13 (d, *J*= 7.6 Hz, 1H), 7.09 (s, 1H), 7.05-7.01 (m, 3H), 4.41 (s, 2H), 2.39 (s, 6H), 1.49 (s, 18 H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 152.3 (s), 145.3 (s), 140.8 (s, 2C), 139.4 (s, 2C), 133.8 (s, 2C), 132.9 (s, 2C), 130.0 (d, 2C), 129.9 (d), 128.0 (d, 2C), 124.4 (d), 121.1 (d), 120.4 (d), 35.1 (t), 32.7 (s, 2C), 31.2 (q, 3C), 31.1 (q, 3C), 21.1 (q, 2C). ^{31}P-NMR (162 MHz, $CDCl_3$) δ 138.4. MS, *m/z* (%): 540 (^{79}Br M^+, 61.9%); HRMS for $C_{29}H_{34}O_3P^{79}Br$, calcd: 540.143, found: 540.142.

6-(3-Bromomethyl-phenoxy)-4,8-di-tert-butyl-2,10-dimethoxy-5,7-dioxa-6-phospha-dibenzo[a,c]cycloheptene (5.47b)



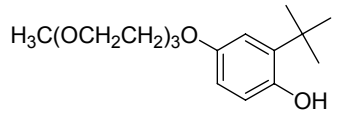
PCl_3 (250 µL, 2.9 mmol) was added dropwise to a solution of NEt_3 (1.6 mL, 11.4 mmol) in dry toluene (5 mL) at 0 °C. Bisphenol **5.62b** (1.0 g, 2.8 mmol) was dried by repeated addition and removal under reduced pressure of toluene (5 mL, 3 times) and then dissolved in toluene (10 mL) and added dropwise to the reaction mixture at 0 °C. The dense yellow reaction mixture was allowed to reach room temperature and it was stirred for 3 h. At this point Et_2O (5 mL) was added, the salts were quickly filtered under a nitrogen atmosphere and the solution obtained was concentrated at room temperature under reduced pressure. The vacuum was then replaced by nitrogen atmosphere and the crude mixture was dissolved in toluene (5 mL). The solution was cooled to 0 °C and NEt_3 (1.6 mL, 11.4 mmol) was added. Benzyl bromide **5.58b** (0.52 g, 2.8 mmol) was dried by repeated addition and removal under reduced pressure of toluene (5 mL, 3 times), dissolved in toluene (10 mL) and added dropwise to the reaction mixture. Stirring at room temperature was continued for 3 h, after addition of Et_2O (5 mL) the salts were filtered and the solvent was removed under reduced pressure. The desired product **5.47b** (50%) was purified by flash column chromatography on silica gel as a white foam (heptanes / ethyl acetate, 95:5). It was stored at -12 °C.
M.p. 41.5-43.8 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29-7.24 (m, 1H), 7.12 (d, *J*= 8.4 Hz, 1H), 7.09 (s, 1H), 7.04-7.00 (m, 1H), 7.02 (d, *J*= 2.8 Hz, 2H), 6.75 (d, *J*= 2.8 Hz, 2H), 4.41 (s, 2H), 3.84 (s, 6H), 1.48 (s, 18H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.8 (s, 2C), 152.3 (s), 142.6 (s, 2C), 141.3 (s), 139.4 (s, 2C), 133.7 (s, 2C), 130.0 (d), 124.4 (d), 121.0 (d), 120.3 (d), 114.4 (d, 2C), 112.9 (d, 2C), 55.6 (q, 2C), 35.4 (t), 32.6 (s, 2C), 31.0 (q, 6C). ^{31}P-NMR (162 MHz, $CDCl_3$) δ 138.4. MS, *m/z* (%): 572 (^{79}Br M^+, 79.4%); HRMS for $C_{29}H_{34}O_5P^{79}Br$, calcd: 572.133, found: 572.133.

1-Chloro-2-[2-(2-methoxy-ethoxy)-ethoxy]-ethane (5.74)



Pyridine (6 mL, 74 mmol) was added to a solution of polyether alcohol **5.73** (10 mL, 62 mmol) in toluene (60 mL). The solution was warmed to 76 °C and $SOCl_2$ (5.4 mL, 74 mmol) was added dropwise and stirring was continued overnight. The reaction mixture was allowed to reach room temperature and it was poured in H_2O (80 mL). The organic layer was separated and the aqueous layer was extracted with toluene. The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product **5.74** was used as such in the following step without further purification.
^{1}H-NMR (300 MHz, CDCl3) δ 3.78-3.70 (m, 2H), 3.70-3.57 (m, 8H), 3.57 -3.50 (m, 2H), 3.37 (s, 3H). ^{13}C-NMR (50 MHz, CDCl3) δ 71.7 (t), 71.3 (t), 70.6 (t), 70.5 (t, 2C), 59.0 (q), 42.6 (t). MS for $C_7H_{15}ClO$, *m/z* (%): 137 (M^+, 4.4%), 91 (100%).

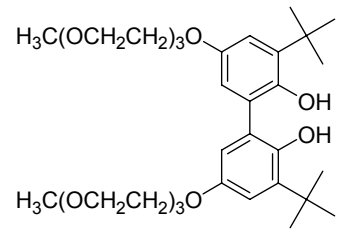
2-tert-Butyl-4-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-phenol (5.60c)



Hydroquinone **5.75** (4.70 g, 0.028 mol) was added in one portion to a mixture of Cs_2CO_3 (7.7 g, 0.024 mol) in CH_3CN (80 mL) and the solution started to color from yellow to dark orange. Polyether chloride **5.74** (4.3 g, 0.024 mol) was then added dropwise. The resulting mixture was stirred under reflux overnight. After cooling the salts were filtered, the solvent was completely removed under reduced pressure and a mixture of EtOAc/H_2O (200 mL) was added. After separation of the two phases, the aqueous layer was extracted with EtOAc, the combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel (ethyl acetate / heptanes, 2:8). The desired product **5.60c** was obtained in 80% yield as a brownish oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.86 (d, *J*= 3.0 Hz, 1H), 6.60 (d, *J*= 8.4 Hz, 1H), 6.53 (dd, *J*= 3.0, 8.4 Hz, 1H), 5.05 (br s, 1H), 4.05-4.98 (m, 2H), 3.84-3.78 (m, 2H), 3.76-3.64 (m, 6H), 3.58-3.53 (m, 2H), 3.37 (s, 3H), 1.37 (s, 9H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 152.4 (s), 148.6 (s), 137.4 (s), 116.7 (d), 114.9 (d), 111.3 (d), 71.9 (t), 70.7 (t), 70.6 (t), 70.5 (t), 69.9 (t), 67.8 (t), 59.0 (q), 34.6 (s), 29.4 (q, 3C).
MS, *m/z* (%): 312 (M^+, 92.4%); HRMS for $C_{17}H_{28}O_5$, calcd: 312.194, found: 312.198.

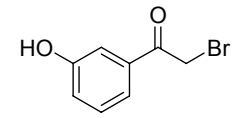
3,3'-Di-tert-butyl-5,5'-bis-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-biphenyl-2,2'-diol (5.62c)



Phenol **5.60c** (2.0 g, 6.4 mmol) was dissolved in acetone (13.5 mL) and the solution obtained was added to a solution containing $K_3[Fe(CN)_6]$ (2.1 g, 6.4 mmol) in H_2O (20.5 mL). As last, an aqueous solution of NaOH (0.26 g, 6.4 mmol, 2.7 mL H_2O) was also added. The heterogeneous reaction mixture was stirred at room temperature for 3 h, after which time $CHCl_3$ (20 mL) was added, the organic layer was separated and the aqueous layer was again extracted with $CHCl_3$ (2 x 25 mL). The combined organic layers were dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification by flash column chromatography on silica gel (ethyl acetate / hexane, 1:1) afforded the desired product **5.62c** (60%) as a thick yellowish oil.

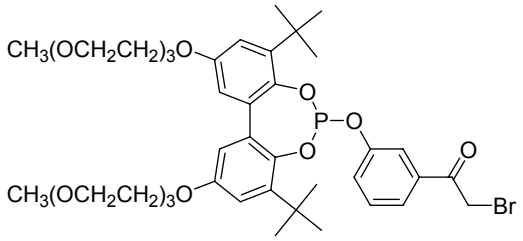
M.p. 76.7-77.2 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.98 (d, *J*= 2.4 Hz, 2H), 6.62 (d, *J*= 3.0 Hz, 2H), 5.09 (s, 2H), 4.11-4.14 (m, 4H), 3.87-3.81 (m, 4H), 3.76-3.61 (m, 12H), 3.56-3.51 (m, 4H), 3.36 (s, 6H), 1.42 (s, 18H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ 152.4 (s, 2C), 146.0 (s, 2C), 138.9 (s, 2C), 123.1 (s, 2C), 116.0 (d, 2C), 112.7 (d, 2C), 72.0 (t, 2C), 70.8 (t, 2C), 70.7 (t, 2C), 70.6 (t, 2C), 69.9 (t, 2C), 68.0 (t, 2C), 59.0 (q, 2C), 35.2 (s, 2C), 29.5 (q, 6C). MS, *m/z* (%): 622 (M^+, 100%); HRMS for $C_{34}H_{54}O_{10}$, calcd: 622.372, found: 622.372.

2-Bromo-1-(3-hydroxy-phenyl)-ethanone (5.72)[180]



A warm solution of 3-hydroxy-acetophenone (**5.71**) (8.0 g, 59 mmol) in $CHCl_3$ (50 mL)[181] was added dropwise to a suspension of $CuBr_2$ (22.3 g, 100 mmol) in EtOAc (50 mL) at reflux. After refluxing for 2.5 h, the color of the reaction mixture changed from dark green to yellow and no evolution of HBr was visible anymore. The reaction mixture was allowed to reach room temperature and H_2O (100 mL) was added. The organic layer was separated, washed with brine, dried over Na_2SO_4 and the solvent was removed under reduce pressure. The desired product **5.72** was obtained as an oil solidifying on standing (85%) after purification by flash column chromatography on silica gel (toluene / ethyl acetate, 20:1).
M.p. 74.1-75.0 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.55-7.51 (m, 2H), 7.38 (t, *J*= 8.0 Hz, 1H), 7.13 (dd, *J*= 1.4, 8.0 Hz, 1H), 5.76 (br, 1H), 4.46 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 192.1 (s), 156.4 (s), 135.1 (s), 130.2 (d), 121.7 (d), 121.4 (d), 115.3 (d), 31.2 (t). MS, *m/z* (%): 214 (^{79}Br M^+, 14.1%); HRMS for $C_8H_7O_2{}^{79}Br$, calcd: 213.963, found: 213.964.

2-Bromo-1-[3-(4,8-di-tert-butyl-2,10-bis-{2-[2-(methoxy-ethoxy)-ethoxy]-ethoxy}-5,7-dioxa-6-phopha-dibenzo[a,c]cyclohepten-6-yloxy)-phenyl]-ethanone (5.47c)



PCl_3 (160 µL, 1.1 eq) was added dropwise at 0 °C to a solution of dry toluene (5 mL) containing NEt_3 (0.9 mL, 4 eq). In the mean time, bisphenol **5.62c** (1.0 g, 1.6 mmol, 1 eq) was dried by repeated addition and removal under reduced pressure of toluene (5 mL, 3 times) and then dissolved in toluene (10 mL) and added dropwise to the reaction mixture at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 3 h. Subsequently, dry Et_2O (5 mL) was added, the salts quickly filtered and the resulting solution was then concentrated under reduced pressure. Toluene (5 mL) was added to the syrup obtained, followed at 0 °C by NEt_3 (0.9 mL, 4 eq). A solution of 3-hydroxy-phenacyl bromide **5.72** (343.6 mg, 1.6 mmol, 1 eq) in toluene (10 mL) was then added dropwise, the reaction mixture was allowed to reach room temperature and stirring was continued for a further 3 h. After this, Et_2O (5 mL) was added and the salts quickly filtered over a plug of silica. The resulting solution was concentrated under reduced pressure and the crude mixture was purified by flash column chromatography on silica gel (ethyl acetate / heptanes, 3:2) yielding the desired product **5.47c** in 72% yield as a colorless syrup, usually stored at -12 °C.
^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J*= 8.0 Hz, 1H), 7.58 (s, 1H), 7.41 (t, *J*= 8.0 Hz, 1H), 7.28 (d, *J*= 8.0 Hz, 1H), 7.04 (d, *J*= 3.2 Hz, 2H), 6.72 (d, *J*= 3.2 Hz, 2H), 4.35 (s, 2H), 4.17-4.11 (m, 4H), 3.89-3.85 (m, 4H), 3.79-3.72 (m, 4H), 3.72-3.63 (m, 8H), 3.58-3.52 (m, 4H), 3.37 (s, 6H), 1.45 (s, 18H). ^{13}C-NMR (100 MHz, $CDCl_3$)

δ 190.4 (s), 155.2 (s, 2C), 152.5 (s), 142.6 (s, 2C), 141.2 (s), 135.3 (s, 2C), 133.5 (s, 2C), 130.1 (d), 126.0 (d), 124.3 (d), 120.6 (d), 115.3 (d, 2C), 113.6 (d, 2C), 71.9 (t, 2C), 70.8 (t, 2C), 70.64 (t, 2C), 70.56 (t, 2C), 69.7 (t, 2C), 67.8 (t, 2C), 59.0 (q, 2C), 35.4 (t), 31.0 (q, 3C), 30.9 (q, 3C), 30.8 (s, 2C). ^{31}P-NMR (162 MHz, $CDCl_3$) δ 137.1. MS for $C_{42}H_{58}BrO_{12}P$, *m/z* (%): 864 (M^+, 0.5%), 786 (100%); HRMS (EI^+), for $C_{42}H_{59}O_{12}P$ calculated on 786 (M^+-Br): 786.375, found: 786.374.

Alkylation of papain (5.77)

An aliquot (100 µL, 1.1 mM) of papain suspension was dissolved in phosphate buffer (9.8 ml, 100 mM, pH 7)[182] containing DTT (100 µL, 100 mM) as reducing agent. The incubation was performed under nitrogen atmosphere at 25 °C for 20 min, while gently stirring. Subsequently, a solution of 3 mL in 1,4-dioxane of **5.47c** (2 mM) was added in portions (6 × 0.5 mL) over a period of 3 h to ensure a high degree of modification of the protein, while gently stirring at 25 °C. The mixture was then filtered (Minisart SRP 15, PTFE-membrane, 0.45 µm) and concentrated (Amicon® Ultra-15, 10K cut off centrifugal filter device, Millipore) with distilled water (3 × 15 mL) in order to exchange solvent and to remove DTT and unreacted phosphite ligand. The resulting concentrated solution (150 µL) was diluted with distilled water to 1 mL.

Activity test[183]

The residual activity of the enzyme was conveniently determined by monitoring the hydrolysis of *Z*-Gly-ONp (**5.67**) as test substrate. This test was performed at various moments during the modification reaction. A sample of the reaction mixture (50 µL) was first diluted in phosphate buffer (950 µL, 100 mM, pH 7). A sample of this diluted solution (90 µL) was added to a quartz cuvette containing phosphate buffer (890 µL, 100 mM, pH 7). Subsequently the substrate was added (20 µL, 2.5 mM, acetone) and its hydrolysis was followed by UV-Vis absorption spectroscopy (λ= 404 nm).

Hydroformylation of styrene (5.79)

The concentrated solution of **5.77** (150 µL, 0.73 mM), obtained after the alkylation step, was diluted with distilled water to 2 mL. The metal precursor $Rh(acac)(CO)_2$ was added and the resulting heterogeneous solution was stirred 30 min. before being filtered (Minisart SRP 15, PTFE-membrane, 0.45 µm). The desired amount of organic solvent was added, followed by styrene (250 µL, 2.17 mmol). The biphasic solution obtained was transferred to a glass vial which was placed in an autoclave. After purging with N_2 (3 × 5 bar) the system was warmed to the desired temperature and pressurized with syngas (H_2:CO, 1:1) and the reaction mixture was vigorously stirred for 16 h. The reaction was stopped by first cooling the autoclave to room temperature and then releasing the syngas pressure. The organic layer was separated and a sample (100 µL) was checked for conversion by ^{1}H-NMR.

2-Phenylpropanal (**5.80a**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.70 (d, *J*= 2.1 Hz, 1H), 7.43-7.12 (m, 5H), 3.64 (q, *J*= 10.5 Hz, 1H), 1.45 (dt, *J*= 10.5, 2.1 Hz, 3H).
3-Phenylpropanal (**5.80b**)[184]: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.83 (t, *J*= 1.5 Hz, 1H), 7.35-7.10 (m, 5H), 2.96 (t, *J*= 7.5 Hz, 2H), 2.77 (tt, *J*= 7.5, 1.5 Hz, 2H).
Enantiomeric excess determination. A sample of the organic solution (300 µL) was added to EtOH (2 mL) followed by an excess of $NaBH_4$ (spatula tip) and the heterogeneous solution was stirred for 90 min. at room temperature.[185] The reaction was quenched by adding H_2O (3 mL) and the mixture was extracted with EtOAc/n-hexane (1:1, 3 times). The combined organic layers were dried over Na_2SO_4 and the solvent removed. A sample of the crude mixture (100 µL) was added to a solution of n-hexane and isopropanol (90:10). A sample of the solution obtained (5-10 µL) was used for the determination of the enantiomeric excess by HPLC.
Conditions: OB-H column; heptanes/isopropanol, 975:25; 0.5 mL/min, λ_{det} 220 nm. Retention time: 2-phenyl-propan-1-ol (from **5.80a**), 17.0/18.3 min.; 3-phenyl-propan-1-ol (from **5.80b**), 20.4 min.

Complexation of 5.77 with $Rh(COD)_2BF_4$ and purification of complex 5.88

A solution of $Rh(COD)_2BF_4$ (8 mM, 8 eq.) in 1,4-dioxane (100 µL) was added to an aqueous solution (1 mL) containing modified papain (**5.77**) (0.1 mM). The resulting yellow solution was gently stirred for 1 h under nitrogen atmosphere at 25 °C. The complex was purified by size exclusion chromatography using desalting columns packed with Sephadex™ G-25 (HiTrap™) connected with an MPLC system (AKTA purifier), or with polyacrylamide Bio-Gel® P-6DG gel (Econo-Pac® 10DG, Biorad). The sample was eluted with phosphate buffer (pH 7, 25 mM). This step allowed to exchange of water with a buffered solution and to remove excess Rh(I) present free in solution.

Hydrogenation of methyl 2-acetamidoacrylate (5.81)

In a typical hydrogenation run, a glass vial was charged with degassed buffer solution (2 mL, phosphate buffer, pH 7, 25 mM) containing the artificial metallo-enzyme complex **5.88** (50 µM) and methyl 2-acetamidoacrylate (**5.81**, 20 mM). The glass vial was placed in an autoclave and after purging with N_2 (3 × 5 bar) the system was pressurized with hydrogen (12 bar) and the reaction mixture was stirred at room temperature for 16 h. The reaction was stopped by release of the H_2 pressure. The resulting mixture was extracted with EtOAc (3 × 5 mL) and the combined organic layers were dried on Na_2SO_4. Conversion was determined by ^{1}H-NMR on a sample of the organic solution. Enantiomeric excess was determined by capillary chiral GC, using the following conditions:
CP Chirasil-L-Val column (25 m × 0.25 mm × 0.25 µm)
Init. Temp.: 110 °C, 12.5 min, 10 °C / min to 160 °C. Tdet/inlet = 250 °C, split ratio 25:1, t_R = 4.09 min, t_S = 4.78 min, t_{SM} = 2.47 min.

ESI-MS measurements

Electrospray mass spectrometry (ESI-MS) was performed on an API3000 mass spectrometer (Applied Biosystems/MDS-SCIEX, Toronto, Canada): a triple quadrupole mass spectrometer supplied with an atmospheric pressure ionization source and a TurboIonSpray interface. The spectra were scanned in the range between *m/z* 1000.0 and 2800.0. The samples were diluted with an aqueous solution of MeOH (85%) containing 0.01% HCOOH.

Kinetics of the inhibition of papain in the presence of cofactor 5.47c

Papain suspension (10 µL, 1.1 mM) and DTT (10 µL, 0.1 M) were added to a sodium phosphate buffer (0.98 mL, 20 mM, pH 6.2) containing NaCl (0.1 M) and EDTA (1 mM). Four solutions were prepared, containing an aliquot of the activation solution (10 µL, 11 µM) diluted with phosphate buffer (0.97 mL). A solution of cofactor **5.47c** in 1,4-dioxane was prepared (0.2 mg/mL, 0.23 mM) and aliquots (5, 10, 15, 20 µL) were added to the solutions containing activated papain. At regular intervals (0, 15, 30 and 45 min.) substrate **5.67** was added (20 µL, 2.5 mM in acetone) and its hydrolysis followed by UV-Vis absorption spectroscopy (404 nm) to measure the residual activity of the enzyme.

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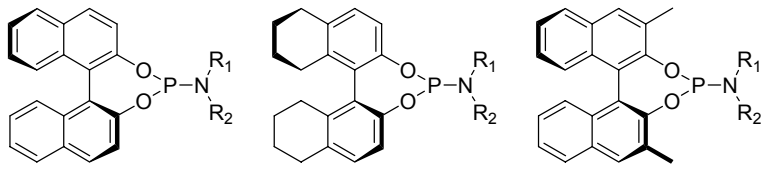
Samenvatting

Vele chemische reacties zouden niet plaats vinden of minder effectief zijn in de afwezigheid van moleculen die we katalysatoren noemen. Wanneer katalysatoren deelnemen aan een chemische reactie levert dat een alternatieve reactieweg op, waarbij de katalysator aan het eind van de reactie onveranderd is. De rol van de katalysator kan worden uitgelegd als: i) stabilisatie van zeer reactieve en instabiele reactanten; ii) de capaciteit om de reactanten in elkaars buurt te brengen en/of in de correcte orientatie; iii) een specifiek reactiepad te bevoordelen ten opzichte van een ongewenst reactiepad.

Overgangsmetaalkatalysatoren behoren tot het meest bestudeerde type katalysatoren. Het complex bestaat uit een overgangsmetaal in het centrum met daarom heen geschikte liganden. Het metaal is verantwoordelijk voor het soort katalytische reactie dat plaats vindt. De ligandmoleculen moduleren de eigenschappen van de katalysator. Door het gebruik van overgangsmetaalkatalysatoren wordt de chemoselectiviteit, regioselectiviteit en enantioslectiviteit van de reactie gestuurd.

Katalytische asymmetrische synthese wordt gedefinieerd als een enantioselectieve transformatie gecontroleerd door een chirale katalysator (in dit geval een metaal gecomplexeerd met chirale liganden). Controle over de absolute stereochemie van de omzetting is één van de meest bestudeerde onderdelen van de homogene katalyse. Het wordt gezien als één van de grootste uitdagingen in de hedendaagse organische chemie. Vandaar dat het gebruik van een katalytische hoeveelheid van een chiraal complex, dat in staat is de chiraliteit van een substraat te sturen tijdens de reactie, een aantrekkelijke benadering is voor het maken van enantiopure verbindingen in vergelijking tot stoichiometrische methoden. Asymmetrische hydrogenering van gefunctionaliseerde achirale alkenen is een van de meest bestudeerde metaalgekatalyseerde omzettingen. Tot voor kort bestonden de meest succesvolle katalysatoren uit Rh(I) met chirale bidentate fosforliganden. De laatste jaren is er echter een succesvolle opleving van het gebruik van monodentate fosforliganden, met inbegrip van BINOL-gebaseerde fosforamidieten (figuur 1).



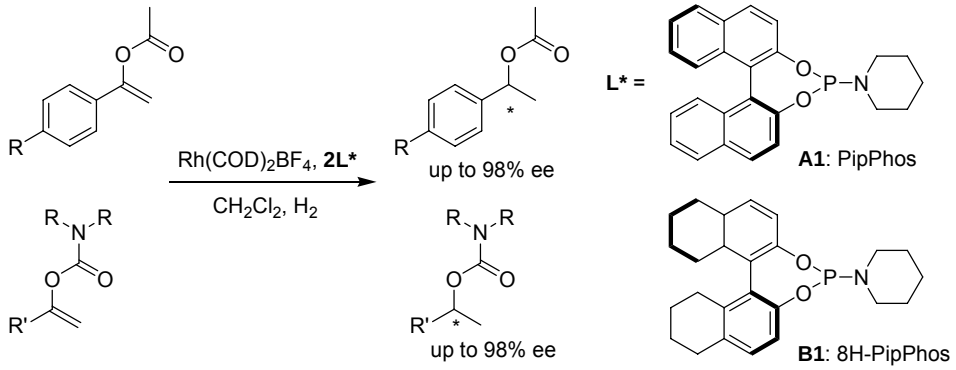
Figuur 1 *Structuren van op BINOL-gebaseerde monodentate fosforamidiet liganden die gebruikt worden in Rh-gekatalyseerde asymmetrische hydrogenering*

Dit proefschrift beschrijft het gebruik van monodentate liganden in homogene Rh-katalyse in nieuwe of minder onderzochte gebieden. Het doel was om i) het gebied van de Rh-gekatalyseerde asymmetrische hydrogenering te verbreden door het

gebruik van fosforamidieten als liganden; ii) het gebruik van deze liganden in de minder bestudeerde Rh-gekatalyseerde asymmetrische hydroformylering te onderzoeken; iii) de mogelijkheden van ligand differentiatie te bestuderen door katalyse en biokatalyse samen te voegen.

Hoofdstuk 1: Een korte inleiding over katalyse wordt gevolgd door een uitleg over Rh-gekatalyseerde hydrogenering en voorbeelden van de doelen die zijn bereikt door gebruik te maken van monodentate fosforamidietliganden. Eén van de voordelen van het gebruik van fosforamidieten is dat ze makkelijk te maken zijn en de structuur eenvoudig kan worden gevarieerd. Het hoofdstuk gaat verder met het belang van liganddifferentiatie en er worden voorbeelden gegeven van interessante benaderingen die gebruikt worden om een grotere verscheidenheid aan katalysatoren te verkrijgen. Daarna wordt een overzicht gegeven van verschillende methodes om met behulp van katalyse belangrijke klassen van enantiopure componenten, zoals α-aminozuren en chirale alcoholen, te maken. Ondanks het belang van hydroformylering in industriële processen is de ontwikkeling van efficiënte katalysatoren voor het maken van chirale aldehyden vrij beperkt. Na een korte inleiding over deze omzetting, worden de meest succesvolle chirale liganden die in asymmetrische hydroformylering gebruikt worden, beschreven.

Hoofdstuk 2: In dit hoofdstuk wordt het succesvolle gebruik beschreven van monodentate fosforamidietliganden in Rh-gekatalyseerde asymmetrische hydrogenering van enol-acetaten en de nieuw ontwikkelde enolcarbamaten als uitgangsstoffen voor de ontwikkeling van chirale alcoholen (figuur 2).

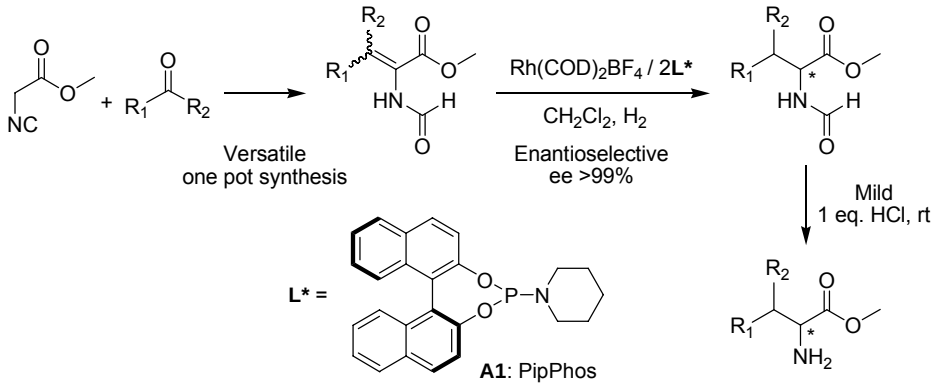


Figuur 2 *Asymmetrische hydrogenering van enol-acetaten en enolcarbamaten*

PipPhos (**A1**) en het overeenkomstige octahydro analoog (**B1**) bleken uitstekende liganden te zijn voor de asymmetrische hydrogenering van aromatische enol-acetaten, aromatische enolcarbamaten en 2-dienylcarbamaten met een uitstekende enantioselectiviteit tot 98%. Een groot effect van de ligandstructuur op de enantioselectiviteit werd waargenomen. De reacties zijn snel (TOF tot 100 h^{-1}, 5 bar H_2). Dit maakt de combinatie van enolcarbamaten en monodentate fosforamidieten zeer concurrerend in vergelijking met de bestaande systemen.

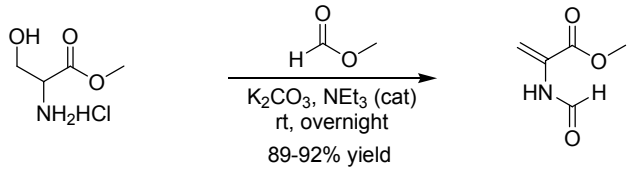
Hoofdstuk 3: Een efficiënte bereiding en Rh-gekatalyseerde asymmetrische hydrogenering van *N*-formyl dehydroamino esters wordt beschreven. De gebruikte

syntheseroute en het gebruik van de formyl beschermgroep maakt de synthese van een grote verscheidenheid van α-aminozuurderivaten mogelijk en biedt het voordeel van een gemakkelijke en milde verwijdering van de formyl beschermgroep (figuur 3).



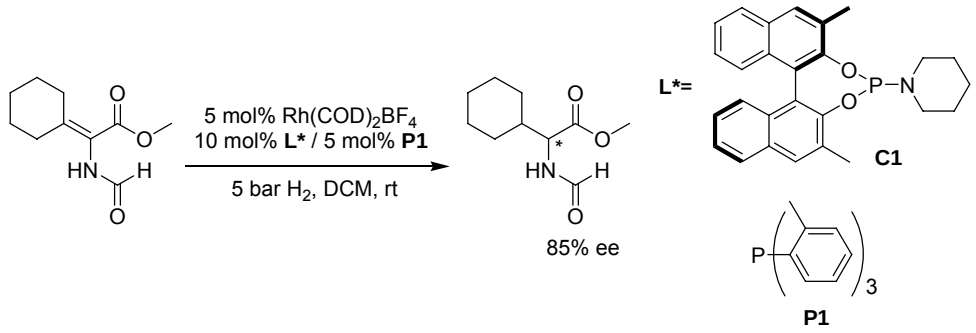
Figuur 3 *Synthese, asymmetrische hydrogenering en ontscherming van N-formyl dehydroamino esters*

De substraten worden gesynthetiseerd door condensatie van methyl isocyanoacetaat met een verscheidenheid aan aldehyden en met cyclohexanon. Enantioselectiviteiten tot 99% ee werden bereikt met asymmetische hydrogenering van de resulterende *N*-formyl dehydroamino esters gebruikmakend van monodentate fosforamidietliganden. PipPhos (A1) blijkt het meest efficiënt te zijn. Een zeer eenvoudige multigramschaal éénstapssynthese van methyl 2-(formamido)acrylaat werd ontwikkeld. (figuur 4). Deze verbinding werd gebruikt in the synthese van methyl 2-(formamido)cinnamaat via een oplosmiddelvrije Heck-reactie.



Figuur 4 *Synthese van methyl 2-(formamido)acrylaat*

Daarnaast werden enantioselectiviteiten tot 85% verkregen in de hydrogenering van methyl 2-(formamido)-3,3-cyclohexylideen acetaat door gebruik te maken van een heterocombinatie van een chiraal fosforamidiet en een achiraal fosfine (figuur 5).



Figuur 5 *Asymmetische hydrogenering van methyl 2-(formamido)-3,3-cyclohexylideen acetaat gebruik makend van een heterocombinatie van liganden*

Hoofstuk 4: De resultaten worden gepresenteerd van een studie, uitgevoerd om inzicht te krijgen in het gebruik van monodentate fosforamidieten als chirale liganden in Rh-gekatalyseerde asymmetrische hydroformylering van styreen en vinylacetaat (figuur 6). Goede activiteiten, chemo-en regioselectiviteiten zijn bereikt. Echter, slechts bescheiden enantioselectiviteiten (tot 27% ee) werden verkregen.

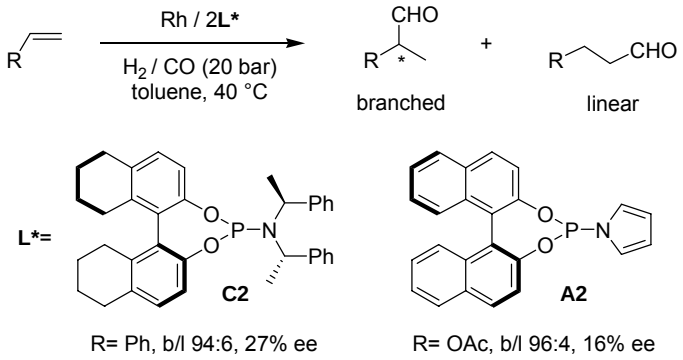
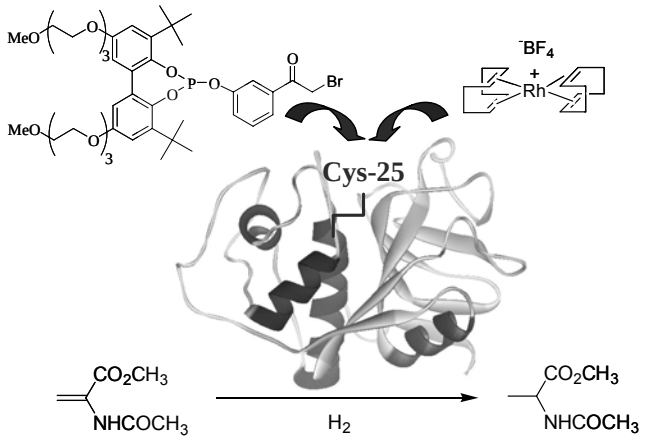


Figure 6 *Asymmetrische hydroformylering van styreen en vinylacetaat*

Hoofdstuk 5: Dit hoofdstuk beschrijft de succesvolle bereiding van een hybride enzymgebonden rodiumkatalysator met een monodentaat ligand (figuur 7). Deze katalysator werd bestudeerd in hydrogenering en hydroformyleringsreakties. Er zijn procedures ontwikkeld voor de binding van het fosforligand aan papaine en de vorming van een complex met $Rh(COD)_2BF_4$. De hydrogenering van methyl 2-(acetamido)acrylaat met het nieuw gemaakte metalloenzym als katalysator, levert het gewenste alaninederivaat met 100% selectiviteit op. Helaas werd er geen enantioselectiviteit verkregen.



Figuur 7 *Het gebruik van een kunstmatig metalloenzym in Rh-gekatalyseerde hydrogenering*

Het gebruik van eiwitten als chirale matrices lijkt een aantrekkelijke en unieke mogelijkheid om de verscheidenheid aan mogelijke katalysatoren te vergroten en ook voor het uitvoeren van op metaal-gebaseerde homogene katalyse in water. Het eiwit zorgt voor de chirale omgeving en de chemokatalysator is verantwoordelijk voor de waargenomen katalytische activiteit. Naast de verscheidenheid aan reeds beschikbare eiwitstructuren, staat deze benadering de mogelijkheid toe gebruik te maken van de voortdurende vooruitgang in de moleculaire biologie voor het maken van grote bibliotheken van eiwitten, nog verder uit te breiden met de combinatoriële benadering voor de identificatie van geschikte katalysatoren voor allerlei soorten omzettingen.

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It was not always fun to be in the office writing my thesis, but I should not complain, I had very nice office-mates: Nathalie, Martin W. and Tibor…even Wesley when he was not there speaking with all his “collaborators”.

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I would also like to remember Gabriella, Suyzi and Natasa together with all the people that I have not mentioned, but that I want to thank for the always pleasant working environment. To Maddalena and Renaud I would like to say: ci vediamo al vostro matromonio! Not much to tell you Ruben, I see you every day…and I am glad.

A special thought for two of the few friends that I had in Groningen outside the university: Charmaine and Esther, I miss our dinners so much.

I would like to mention my two paranimfen Francesca and Wendy. Thank you for being good friends. Wendy was my first Dutch friend and Francesca my first Italian friend in the Netherlands. You are both very close to my heart, but when I see one of you I cannot see the other! Wendy, thank you for translating my summary into dutch. Francesca, grazie per esserci sempre stata quando avevo bisogno di sfogarmi.

Dear Richard, do you see? There can be friendship at work!

Un grazie speciale va alla mia mamma e al mio papa`. Lo so quanto e` stato difficile per voi in questi anni, ma vi ringrazio per aver cercato di capire ed accettare le mie scelte. Quello che sono lo devo a voi e ai vostri sacrifici.

Dear Tim, my last thought is for you. I could not have done all this without you and your support. Thank you for being always on my side and believing in me even when I doubted myself. Sorry for all the week-ends spent in the train between Groningen and Maastricht, that time is over!